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Transplantation of Islet Organoids into Brown Adipose Tissue in a Diabetic Mouse Model

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Abstract

Pancreatic islet transplantation is a promising cell replacement therapy for patients with type 1 diabetes (T1D), an autoimmune disease that destroys insulin-producing islet β cells. However, the shortage of donor pancreatic islets significantly limits the widespread use of this strategy as a routine therapy. Pluripotent stem cell-derived insulin-producing islet organoids present a promising alternative β cell source for T1D patients. One critical challenge is the lack of vascularization in islet organoids, making it essential to investigate vascularized transplantation sites to support their survival. Brown adipose tissue (BAT) is well vascularized and secretes active cytokines, facilitating islet organoid survival. Thus, BAT represents a promising transplantation site for islet organoids, making it an ideal location to support cell replacement therapies and improve treatment approaches for T1D. Here, we describe the methods for transplanting human-induced pluripotent stem cell (iPSC)-derived islet organoids into the BAT of a mouse model.

Key words Type 1 diabetes, Stem cell differentiation, Pluripotent stem cell, β cells, Islet organoids, Islet organoids transplantation, Brown adipose tissue (BAT)

1 Introduction

Type 1 diabetes (T1D) is an autoimmune disorder marked by chronic hyperglycemia due to the gradual destruction of insulin-producing islet β cells [1, 2]. While exogenous insulin injections effectively lower blood glucose levels in T1D patients, they do not restore the physiological regulation of blood glucose, as they are merely symptomatic treatments. Consequently, T1D patients remain at risk for long-term complications, including severe hypoglycemia, chronic retinopathy, nephropathy, and cardiovascular disease [3, 4].

Pancreatic islet transplantation is a promising strategy for restoring physiological insulin secretion in T1D patients [5, 6]. However, the widespread use of this therapy is limited by the scarcity of cadaveric islets, prompting research into alternative islet sources. Additionally, the high risk of host immune rejection requires patients who undergo islet transplantation to remain on lifelong immunosuppressive medication.

Research has increasingly focused on pluripotent stem cell-derived insulin-producing cells as an alternative for T1D treatment, particularly in the form of insulin-producing islet organoids [7, 8]. These organoids offer solutions to the current challenges, as pluripotent stem cells provide an unlimited supply. Moreover, they can be developed autologously, significantly reducing the risk of allogenic immune rejection.

The microenvironment of the transplantation site significantly influences the survival and function of transplanted islet organoids [9]. To date, several studies have explored the subcutaneous transplantation of encapsulated islet organoids in T1D patients. Recent clinical trials have shown that subcutaneous transplantation of encapsulated human embryonic stem cell (hESC)-derived pancreatic endoderm cells resulted in detectable levels of glucose-stimulated C-peptide (33–99 pmol) over a 12- to 24-month period [8, 10, 11]. However, insulin production remained below therapeutic levels for these patients. These findings have initiated the search for other potential transplantation sites that might improve islet organoid survival and enhance graft functionality post-transplantation [12]. A crucial factor for optimizing the survival of islet organoids after transplantation is locating a site with abundant vascularization and cytokine activity to ensure adequate oxygen and nutrient supply [13, 14]. Brown adipose tissue (BAT), a thermogenic tissue that generates heat in response to cold, is highly vascularized and innervated by the sympathetic nervous system [15–18]. Research indicates that BAT could serve as a promising site for islet or islet organoids transplantation, as it may reduce immune rejection, minimize inflammation in adipose tissue, and maintain BAT functionality [19–21]. Here, we present a detailed methodological protocol for transplanting human iPSC-derived islet organoids into the BAT of diabetic mice.

2 Materials

2.1 Cell Culture

1. Human-induced pluripotent stem cells (hiPSCs): hiPSC-L1, AICS-0037-172 (Coriell Institute for Medical Research, alias AICS) [22].
2. Essential 8 Flex medium (Thermo Fisher Scientific, Waltham, MA) containing 1% penicillin/streptomycin (P/S, Gibco, Billing MT), ReLeSR passaging reagent (STEMCELL Technologies).
3. Matrigel (Corning 354230, Corning, NY).
4. Six-well ultra-low attachment tissue culture plate (Corning, Corning, NY).

5. CO_2 incubator (Thermo Scientific), Class II type A2 biological safety cabinet (Thermo Scientific).

2.2 Pancreatic Islet Organoid Differentiation

1. hiPSCs and Essential 8 Flex medium (Thermo Fisher Scientific) containing 1% penicillin/streptomycin (pen/strep, P/S, Gibco, Billings, MT).
2. Accutase (Innovative Cell Technologies, San Diego, CA).
3. 96-well ultra-low attachment plate (Corning, Corning, NY).
4. CO_2 incubator (Thermo Scientific), Class II type A2 biological safety cabinet (Thermo Scientific).
5. RPMI (Gibco, Thermo Fisher Scientific), DMEM (Gibco, Thermo Fisher Scientific), CMRL 1066 Supplemented (Mediatech, Manassas, VA), and 1× phosphate-buffered saline (PBS) (Sigma-Aldrich, Milwaukee, WI).
6. MCDB 131 (CellGro, Lincoln, NE), recombinant human Activin A protein (Activin A, R&D Systems), glucose (MilliporeSigma), sodium bicarbonate (MilliporeSigma), BSA (HyClone, Cytiva, Marlborough, MA), ITS-X (Invitrogen, ThermoFisher, Waltham, MA), GlutaMAX (Invitrogen, Thermo Fisher), vitamin C (MilliporeSigma), heparin (MilliporeSigma), 10% fetal bovine serum (HyClone, Cytiva), Chir99021 (Stemgent), KGF (Peprotech), Sant1 (Sigma), RA (Sigma), LDN193189 (Sigma), PdBU (EMD Millipore), XXI (EMD Millipore), Alk5i II (Axxora), T3 (EMD Millipore), Betacellulin (Thermo Fisher Scientific).
7. Corning® bottle-top vacuum filter system (CLS431097).
8. Anti-insulin antibody (Abcam), anti-glucagon antibody (Abcam).
9. Insulin Human ELISA kit (Abcam, ab100578).

2.3 Diabetes Induction and Blood Glucose Monitoring for Diabetic Mice

1. Twelve-week-old female NOD/SCID mice (Jackson Laboratory, Bar Harbor, ME).
2. Streptozotocin (STZ, Sigma-Aldrich).
3. Sodium citrate (Sigma-Aldrich).
4. Citric acid (Sigma-Aldrich).
5. Deionized water.
6. Metene TD-4116 blood glucose monitor kit.
7. Glucometer strips.

2.4 Islet Organoid Transplantation into the BAT of a Diabetic Mouse Model

1. Diabetic mice (12-week-old female NOD/SCID mice, injected with STZ and blood glucose higher than 250mg/dL).
2. Animal anesthesia system Isotec 4 (Surgivet/Anesco).
3. Surflo® winged infusion set 25 Gx3/4″ (TERUMO).

4. Polyethylene tubing I.D. 0.76 mm (0.030″), O.D. 1.22 mm (0.048″) (SCI, BB31695-PE/4).

3 Methods

3.1 Cell culture

1. Culture hiPSC-L1 cells in Essential 8 Flex medium with 1% penicillin/streptomycin on 6-well plates precoated with growth factor-reduced Matrigel in a 37 °C, 5% CO_2 incubator.
2. Once the cells reach 70–80% confluency, passage them into a new 6-well plate using ReLeSR passaging reagent.

3.2 Islet Organoid Differentiation

1. Prepare the differentiation media according to the following formulations [23]:
   - Stage 1 Medium: Combine 500 mL MCDB 131 with 0.22 g glucose, 1.23 g sodium bicarbonate, 10 g BSA, 10 μL ITS-X, 5 mL GlutaMAX, 22 mg vitamin C, and 5 mL penicillin/streptomycin (P/S) solution.
   - Stage 2 Medium: Combine 500 mL MCDB 131 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 solution.
   - Stage 3 Medium: Combine 500 mL MCDB 131 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 solution.
   - Stage 4 Medium: Combine 500 mL MCDB 131 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 solution, and 5 mg heparin.
   - Stage 5 Medium: Prepare CMRL 1066 supplemented with 10% fetal bovine serum, and 5 mL P/S solution.
2. Sterilize the culture media using a bottle-top vacuum filter system according to the manufacturer's instructions.
3. Dissociate confluent hiPSC-L1 cultures into small cell clusters using Accutase.
4. Dispense the dissociated hiPSC-L1 cells into 96-well low-adherence round-bottom plates using an 8-channel pipettor to form cell spheres (*see* **Note 1**).
5. Treat the cell spheres with the previously prepared stage 1–5 culture media [23] (*see* **Note 2**).
6. After completing the five stages of differentiation, evaluate the differentiated islet organoids for islet-specific markers, such as

insulin and glucagon, using qPCR and immunofluorescence techniques [7].

7. Conduct a glucose-stimulated insulin secretion (GSIS) assay on the differentiated islet organoids. Begin by washing one hundred islet organoids (differentiated for 28–30 days) in 1 ml of low-glucose (2.8 mM) Krebs buffer (KRB) twice to remove any residual insulin. Incubate the organoids in 1 mL of 2.8 mM glucose KRB for 30 min at 37 °C in a cell culture incubator, and then collect the supernatant. Wash the organoids twice more with 2.8 mM glucose KRB, and then incubate them in 1 mL of high-glucose (28 mM) Krebs buffer for 30 min and collect the supernatant. Measure human insulin levels using the Insulin Human ELISA kit [24, 25] (*see* **Note 3**).

3.3 Preparation of STZ Solution and Induction of the Diabetic Mouse Model

1. Prepare a 0.1 M sodium citrate solution by dissolving 14.71 g of sodium citrate in 200 mL of deionized water.
2. Prepare a 0.1 M citric acid solution by dissolving 20.1 g of citric acid in 200 mL of deionized water.
3. Combine the 0.1 M sodium citrate and 0.1 M citric acid solutions and adjust the pH to 4.5 using the 0.1 M citric acid solution.
4. Dissolve STZ in the acidic citrate buffer (0.1 M) solution and store it in the dark by wrapping the container in aluminum foil. Keep the solution at 4 °C for 2 h post-dissolution to allow for anomer equilibration [26] (*see* **Note 4**).
5. House 12-week-old female NOD/SCID mice under a 12-h light/dark schedule at a temperature of 22–23 °C, providing standard lab chow and acidified water freely.
6. Induce diabetes in the mice through an intraperitoneal injection of STZ at a dosage of 200 mg/kg of body weight.
7. Confirm the onset of diabetes by observing weight loss, increased urine output (polyuria), and blood glucose levels exceeding 250 mg/dL in at least two separate tests.

3.4 Islet Organoid Transplantation

1. Fill the winged infusion set with PBS, introducing no air bubbles into the tubing.
2. Allow the islet organoids to settle at the bottom of the plate. Using a straight cut-tip pipette, carefully draw the pellet into a P200 tip (set to 100 μL) and transfer the tip to the adapter of the winged infusion set, depositing the pellet inside.
3. Secure the winged infusion set to the side of the hood, taping it to the hood wall with the needle side of the tube clipped to prevent leakage. This setup will allow the islet organoids to remain concentrated in the adapter.

4. Attach a 1 mL syringe to the adapter of the winged infusion set and gently push the islet organoids forward into the tubing.
5. Anesthetize the mouse with 2% isoflurane and shave the back of the diabetic mouse. Clean the mouse's skin using a Povidone Iodine swab (from the center outward) and then wipe the area with an ethanol swab.
6. Make a small incision at the scapular region to expose the underlying white adipose tissue. Fold back the white fat to reveal the large, bifurcated scapular brown adipose tissue (BAT) depot.
7. Using polyethylene tubing connected to a butterfly needle, infuse the islet organoid suspension (400 IEQ) into the left lobe of the BAT (*see* **Note 5**).
8. After infusion, remove the syringe and return the white fat to cover the BAT.
9. Close the incision using sutures and skin staples [21].
10. Monitor the recipient mice' blood glucose levels twice daily using the Metene TD-4116 blood glucose monitor kit.

4 Notes

1. Using an 8-channel pipettor, distribute the dissociated hiPSC-L1 cells into 96-well low-adherence round-bottom plates, with each well containing 150 μL of cell suspension in Essential 8 Flex Medium supplemented with 10 μM Y27632. Centrifuge the plate at 300 g for 5 min to encourage cell aggregation and formation of cell spheres.
2. Begin differentiation 48 h after the cell spheres have formed, following the medium schedule below:
   - Day 1: Stage 1 medium with 100 ng/mL Activin A and 3 μM Chir99021.
   - Day 2: Stage 1 medium with 100 ng/mL Activin A.
   - Days 4-6: Stage 2 medium with 50 ng/mL KGF.
   - Day 7: Stage 3 medium with 50 ng/mL KGF, 0.25 μM Sant1, 2 μM RA, 200 nM LDN193189, and 500 nM PdBU.
   - Day 8: Stage 3 medium with 50 ng/mL KGF, 0.25 μM Sant1, 2 μM RA, and 500 nM PdBU.
   - Days 9–13: Stage 3 medium with 50 ng/mL KGF, 0.25 μM Sant1, and 100 nM RA.
   - Days 14–16: Stage 4 medium with 0.25 μM Sant1, 100 nM RA, 1 μM XXI, 10 μM Alk5i II, 1 μM T3, and 20 ng/mL Betacellulin.

- Days 18–20: Stage 4 medium with 25 nM RA, 1 μM XXI, 10 μM Alk5i II, 1 μM T3, and 20 ng/mL Betacellulin.
- Days 21–35: Stage 5 medium with 10 μM Alk5i II, 1 μM T3, and 20 ng/mL Betacellulin, changing the medium every other day.

3. Normalize human insulin levels by cell counts. Disperse the islet organoids into single cells using Accutase and count them with a cell counter.
4. Note that the STZ solution is stable only within a pH range of 4.2–4.5 and is unstable in an unbuffered aqueous solution. It is recommended that the STZ solution be prepared and injected immediately to induce diabetes [27]. The citrate buffer for STZ should be freshly prepared or stored in aliquots at −20 °C [28, 29].
5. Slowly inject the syringe while observing the movement of the islet organoids into the left lobe of the BAT. Once all organoids are delivered into the left lobe, gently remove the syringe and apply light pressure with a dry swab on the injection site.

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References

1. DiMeglio LA, Evans-Molina C, Oram RA (2018) Type 1 diabetes. Lancet 391(10138): 2449–2462
2. Popoviciu MS et al (2023) Type 1 diabetes mellitus and autoimmune diseases: a critical review of the association and the application of personalized medicine. J Pers Med 13(3)
3. Daneman D (2006) Type 1 diabetes. Lancet 367(9513):847–858
4. Sherr JL et al (2024) Severe hypoglycemia and impaired awareness of hypoglycemia persist in people with type 1 diabetes despite use of diabetes technology: results from a cross-sectional survey. Diabetes Care 47(6):941–947
5. Kahanovitz L, Sluss PM, Russell SJ (2017) Type 1 diabetes – a clinical perspective. Point Care 16(1):37–40
6. Akil AA et al (2021) Diagnosis and treatment of type 1 diabetes at the dawn of the personalized medicine era. J Transl Med 19(1):137
7. Sun A et al (2021) 3D in vivo magnetic particle imaging of human stem cell-derived islet organoid transplantation using a machine learning algorithm. Front Cell Dev Biol 9: 704483
8. Keymeulen B et al (2024) Encapsulated stem cell-derived beta cells exert glucose control in patients with type 1 diabetes. Nat Biotechnol 42(10):1507–1514
9. Saber N et al (2023) The impact of different implantation sites and sex on the differentiation of human pancreatic endoderm cells into insulin-secreting cells in vivo. Diabetes 72(5): 590–598
10. Shapiro AMJ et al (2021) Insulin expression and C-peptide in type 1 diabetes subjects implanted with stem cell-derived pancreatic endoderm cells in an encapsulation device. Cell Rep Med 2(12):100466
11. Ramzy A et al (2021) Implanted pluripotent stem-cell-derived pancreatic endoderm cells secrete glucose-responsive C-peptide in patients with type 1 diabetes. Cell Stem Cell 28(12):2047–2061 e5

12. Wang S et al (2024) Transplantation of chemically induced pluripotent stem-cell-derived islets under abdominal anterior rectus sheath in a type 1 diabetes patient. Cell 187 (22):6152–6164
13. Nalbach L et al (2021) Improvement of islet transplantation by the fusion of islet cells with functional blood vessels. EMBO Mol Med 13(1):e12616–n/a
14. Cayabyab F, Nih LR, Yoshihara E (2021) Advances in pancreatic islet transplantation sites for the treatment of diabetes. Front Endocrinol (Lausanne) 12:732431
15. Gunawardana SC, Piston DW (2012) Reversal of type 1 diabetes in mice by brown adipose tissue transplant. Diabetes 61(3):674–682
16. Sarkanen JR et al (2012) Human adipose tissue extract induces angiogenesis and adipogenesis in vitro. Tissue Eng Part A 18(1-2):17–25
17. Ikeda K, Maretich P, Kajimura S (2018) The common and distinct features of brown and beige adipocytes. Trends Endocrinol Metabol 29(3):191–200
18. White JD, Dewal RS, Stanford KI (2019) The beneficial effects of brown adipose tissue transplantation. Mol Aspects Med 68:74–81
19. Kepple JD et al (2022) Islet transplantation into brown adipose tissue can delay immune rejection. JCI Insight 7(4)
20. Xu K et al (2020) Brown adipose tissue: a potential site for islet transplantation. Transplantation 104(10):2059–2064
21. Sun A et al (2024) Brown adipose tissue as a unique niche for islet organoid transplantation: insights from in vivo imaging. Transplant Direct 10(7):e1658
22. Lewis-Israeli YR et al (2021) Self-assembling human heart organoids for the modeling of cardiac development and congenital heart disease. Nat Commun 12(1):5142
23. Pagliuca FW et al (2014) Generation of functional human pancreatic β cells in vitro. Cell 159(2):428–439
24. Slepchenko KG, Corbin KL, Nunemaker CS (2019) Comparing methods to normalize insulin secretion shows the process may not be needed. J Endocrinol 241(2):149–159
25. Sun A et al (2023) In vivo bioluminescence for the detection of the fate of pancreatic islet organoids post-transplantation. Methods Mol Biol 2592:195–206
26. de la Garza-Rodea AS et al (2010) Anomer-equilibrated streptozotocin solution for the induction of experimental diabetes in mice (Mus musculus). J Am Assoc Lab Anim Sci 49(1):40–44
27. Mythili MD et al (2004) Effect of streptozotocin on the ultrastructure of rat pancreatic islets. Microsc Res Tech 63(5):274–281
28. Tesch GH, Allen TJ (2007) Rodent models of streptozotocin-induced diabetic nephropathy. Nephrology (Carlton) 12(3):261–266
29. Ghasemi A, Jeddi S (2023) Streptozotocin as a tool for induction of rat models of diabetes: a practical guide. Excli J 22:274–294