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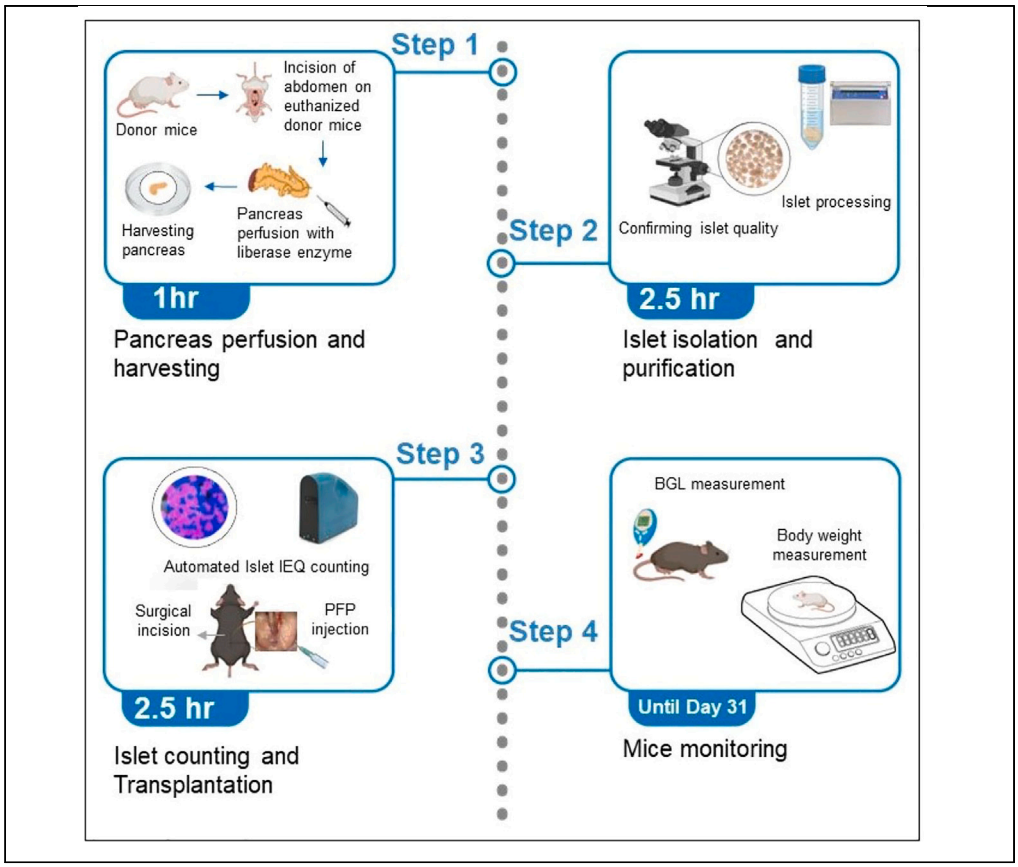
Protocol for transplanting pancreatic islets into the parametrial fat pad of female mice



Although the male epididymal fat pad is an effective site for islet transplantation, females lack this tissue. Here, we present a protocol to assess the parametrial fat pad (PFP) adjacent to the uterine horn in females as an alternative site for islet transplantation. We describe steps for islet isolation from the pancreas, counting, transplantation into PFP, and monitoring for engraftment. Transplantation into PFP is minimally invasive, time efficient, and supports long-term engraftment of syngeneic islets and rejection of allogeneic islets.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Mebrahtu G. Tedla, Nathaniel Wright, Esma S. Yolcu, Yadong Wang, Haval Shirwan

haval.shirwan@health.missouri.edu

Highlights

Protocol for establishing parametrial fat pad as a site for islet transplantation

Induction of diabetes using streptozotocin

Detailed steps for islet isolation and implantation into PFP of diabetic recipients

Assessing islet engraftment by monitoring blood glucose levels

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Protocol for transplanting pancreatic islets into the parametrial fat pad of female mice

Mebrahtu G. Tedla,[1,2,3,5] Nathaniel Wright,[4] Esma S. Yolcu,[1,2,3] Yadong Wang,[4] and Haval Shirwan[1,6,*]

[1]Department of Pediatrics, Ellis Fischel Cancer Center, School of Medicine, University of Missouri, Columbia, MO 65211, USA

[2]Department of Molecular Microbiology and Immunology, Ellis Fischel Cancer Center, School of Medicine, University of Missouri, Columbia, MO 65211, USA

[3]NextGen Precision Health Institute, Ellis Fischel Cancer Center, School of Medicine, University of Missouri, Columbia, MO 65211, USA

[4]Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY 14853, USA

[5]Technical contact

[6]Lead contact

*Correspondence: haval.shirwan@health.missouri.edu
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SUMMARY

Although the male epididymal fat pad is an effective site for islet transplantation, females lack this tissue. Here, we present a protocol to assess the parametrial fat pad (PFP) adjacent to the uterine horn in females as an alternative site for islet transplantation. We describe steps for islet isolation from the pancreas, counting, transplantation into PFP, and monitoring for engraftment. Transplantation into PFP is minimally invasive, time efficient, and supports long-term engraftment of syngeneic islets and rejection of allogeneic islets.

For complete details on the use and execution of this protocol, please refer to Zhang et al. (2022).[1]

BEFORE YOU BEGIN

The protocol below describes the specific steps for islet transplantation into the parametrial fat pad (PFP) in female mice. Transplantation into PFP will facilitate studies in settings where there is a high prevalence of the disease among females, such as autoimmune type 1 diabetes.

Institutional permissions

All experiments and animal care and handling protocols have been implemented in line with NIH policies for the use of animals and approved by the Institutional Animal Care and Use Committee at the University of Missouri, Columbia, MO. Institutional approval for the use of animals is required for the implementation of this protocol.

Mouse pancreatic islet isolation

Timing: 25 min

1. Set the temperature for a water bath at 37°C and centrifuge at 4°C.
2. Prepare a coating buffer solution (30% of FBS in HBSS with calcium and magnesium).
3. Prepare islet washing buffer solution.
4. Dissolve Liberase TL (0.18 mg/mL) in Ca^{2+} and Mg^{2+}-free HBSS.
5. Prepare a complete islet culture medium.
6. Prepare iDTZ islet staining solution.
7. Prepare 25% Ficoll solution.

8. Prepare sterilized surgical instruments.
9. Prepare mice for surgical procedures.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Ammonium hydroxide solution	Sigma-Aldrich	Cat#338818
2 ME (β-mercaptoethanol)	Sigma	Cat#M-7522
Dithizone (DTZ)	Sigma	Cat#194832
DMSO	ATCC	Cat#4-X
DPBS	Sigma	Cat#D8537
FBS	Gibco	Cat#16000044
Ficoll type 400 DL	Sigma	Cat#F-9378
0.9% Sodium chloride injection USP	B. Braun Medical Inc.	Cat#S8004-5264
HBSS (w/o: calcium, magnesium, phenol red)	Gibco	Cat#14175095
HBSS (with calcium, magnesium, no phenol red)	Gibco	Cat#14025092
HEPES (1 M)	Gibco	Cat#15630080
Liberase TL research grade	Roche	Cat#5401020001
L-glutamine	Gibco	Cat#25030-081
Methanol	Sigma-Aldrich	Cat#34860
Penicillin-Streptomycin (10,000 U/mL-10,000 μg/mL)	Gibco	Cat#15140122
RPMI 1640 medium, no glutamine	Gibco	Cat#21870076
2,2,2-Tribromoethanol (Avertin)	Sigma-Aldrich	Cat#75809
Ketamine	Sigma-Aldrich	Cat#1867669
Xylene	Sigma-Aldrich	Cat#1330207
Collagenase NB1	Nordmark	Cat#S1745602
Streptozotocin (STZ)	Sigma-Aldrich	Cat#S0130
Experimental models: Mouse strains		
BALB/cJ mice (male and female animals; 8–12 weeks old)	The Jackson Laboratory	Strain #000651
C57BL/6J mice (male and female animals; 8–12 weeks old)	The Jackson Laboratory	Strain #000664
NOD mice (female mice; 8–12 weeks old)	The Jackson Laboratory	Strain #001976
Other		
Absorbable gelatin sponge	Ethicon	Cat#1972
1.5-inch bulldog clamp	Fine Science Tools	Cat#1805135
Falcon 17 × 100 mm, 14 mL round polypropylene bottom test tubes	Corning Life Sciences	Cat#352059
CO_2 incubator	Thermo Scientific	Cat#Forma Series II HEPA Class 100
3 mL syringe	BD	Cat#309657
100 mm Petri dish	Fisherbrand	Cat#FB0875712
50 mL tubes	Fisherbrand	Cat#05-539-13
60 mm Petri dish	Fisherbrand	Cat#FB0875713A
Centrifuges	Thermo Scientific	Cat#Sorvall X Pro Series
Small curved surgical scissors	Electron Microscopy Sciences	Cat#729355
General-purpose water bath	Fisher Scientific	Cat#FSGPD20
Gel-loading tips, 1–200 μL	Fisherbrand	Cat#02-707-81
Portable balances	OHAUS	Cat#SCOUT SPX
SURFLO winged infusion set	Terumo	Cat#SV*25BLS
1 mL syringe	BD	Cat#309623
Nylon mesh filters, 500 μm,	Tisch Scientific	Cat#ME17264
0.2 μm bottle top filter	Thermo Fisher Scientific	Cat#596-4520
EVENCARE G2 glucose stripes	Medline	Cat#MPH1550
EVENCARE G2 blood glucose meter	Medline	Cat#MPH1540NV
Ultra-fine point tweezers	Fisherbrand	Cat#12000122
Surgical scissors	Fine Science Tools	Cat#9140012
30G x 1/2″ needle	EXELINT International	Cat#26437

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Automatic islet cell counter	ICC4-115/230	Biorep
Ward's benchtop counter (6 key)	470311-184	VWR International
Inverted cell culture microscope	Cat#CKX53	Olympus
Trinocular lab compound microscope	M837SL-C90U	OMAX
7X-45X stereo zoom inspection industrial microscope	Cat#SM-1BN	AmScope
Trinocular stereo zoom microscope + 144 direction adjustable LED ring light	SM-3TZZ-144A-5M3-B	Microscope Central
7X-90X binocular stereo boom microscope + ring light	Cat#SM-4BY-FRL	AmScope
7X-45X Simul-focal stereo zoom microscope on boom stand + fiber optic ring light	MEDZ250390	Medz Industrial

MATERIALS AND EQUIPMENT

Coating buffer solution (30%)

Reagent	Final concentration	Amount
HBSS (supplemented with Ca^{2+} and Mg^{2+})	N/A	35 mL
Fetal bovine serum	1%	15 mL
Total	**N/A**	**50 mL**

Islet wash solution

Reagent	Final concentration	Amount
HBSS (supplemented with Ca^{2+} and Mg^{2+})	N/A	485 mL
HEPES (1 M)	20 mM	10 mL
Fetal bovine serum	1%	5 mL
Total	**N/A**	**500 mL**

0.18 mg/mL liberase LT solution

- Dissolve 5 mg of Liberase TL in 27.7 mL of HBSS (Ca^{2+} and Mg^{2+}-free).
- Keep the enzyme solution on ice for a minimum of 10–20 min until dissolved completely and mix the solution promptly a couple of times before use.

Note: Liberase solution should be prepared fresh and kept on ice throughout the pancreas harvesting process and used within 1 h. One perfusion requires 3 mL of the solution. The required Liberase enzyme volume should be determined based on the total number of mice to be used for islet isolation.

Complete islet culture medium

Reagent	Final concentration	Amount
RPMI-1640	N/A	43.5 mL
Penicillin-Streptomycin (10,000 U/mL & 10,000 µg/mL)	100 U/mL and 100 µg/mL	0.5 mL
Fetal Bovine Serum	10%	5 mL
2 ME (β-mercaptoethanol) (50 mM)	50 µM	0.5 µL
L-Glutamine (200 mM)	2 mM	0.5 mL
Total	**N/A**	**50 mL**

Note: All solutions should be prepared fresh and kept on ice until islet processing is complete. Complete islet culture media should be stored at 4°C and should not be used after one week.

iDTZ solution

The iDTZ solution for islet staining and counting is prepared according to the protocol provided by the manufacturer[1] and enumerated as follows:

- Dissolve 100 mg of iDTZ in 20 mL dimethyl sulfoxide (DMSO).
- Add 30 mL HBSS into the solution.
- Filter the solution using a 0.2 or 0.4 μm bottle top filter and add 50 mL HBSS.
- Store at 4°C.

Note: Islet staining using iDTZ varies from sample to sample. Islets must be stained for a minimum of 10 min for optimum staining and accurate counting using the ICC4 software,

Ficoll stock solutions

- Weight 25 g of Ficoll and dissolve in 75 mL HBSS (supplemented with Ca^{2+} and Mg^{2+}).

Note: To completely dissolve Ficoll, use a magnetic stirrer and keep the solution overnight at room temperature. Sterilize the solution by passing through a 0.2 μm bottle top filter (Thermo Fisher Scientific) and keep at 4°C in the dark until use.

- Prepare the following Ficoll solutions.
- For 23%: add 23 mL of the 25% Ficoll and 2 mL HBSS.
- For 20.5%: add 20.5 mL of the 25% Ficoll and 9 mL HBSS.
- For 11%: add 11 mL of the 25% Ficoll and 14 mL HBSS.

Note: Keep the solutions in the dark at 4°C and bring them to the room temperature before use.

STEP-BY-STEP METHOD DETAILS

Induction of diabetes by streptozotocin

Timing: 25 min

We describe a protocol for diabetes induction using streptozotocin (STZ) through tail vein, monitoring mice for blood glucose (BG) to confirm diabetes and body weight for overall health assessment. Animals over 400 mg/dL BG were treated with insulin to control the adverse effects of high BG and expedite post-surgical recovery. STZ can be administered intraperitoneally, but this procedure is less efficient in the induction of diabetes and may require more mice to obtain adequate diabetic numbers for the intended study.

1. Four days before the transplantation, measure the body weight of prospective graft recipients.
2. Prepare streptozotocin (STZ) solution (20 mg/mL) in 0.9% saline.

Note: STZ in solution is labile and fresh solution should be prepared 15 min before injections.

Note: Gentle vortexing is recommended to dissolve STZ.

3. Inject STZ solution (200 mg/kg) i.v. using a 27G needle on day -4 before islet transplantation.

Note: To facilitate tail vein injection, warm mice in a commercially available warming box, a brass restrainer, or a warm water circulating pad set beneath the cage for 5–10 min to dilate the vein. Avoid air bubbles in the needle while injecting STZ into the tail vein as this leads to air

embolism and sudden death. In addition, administer the solution into the vein carefully and slowly to avoid local irritation and often death because of shock.

Note: Intraperitoneal (i.p.) injection is an alternative to i.v. injection of STZ. However, unintentional distribution of the drug into the colon or subcutaneous area may enhance morbidity or reduce the diabetogenic impact on the recipient.[2]

Furthermore, compared to i.p., administration of STZ i.v. achieves consistent diabetes with limited morbidity and mortality.

Measure the blood glucose level (BGL) and body weight on day -1 and day 0 (the day of transplantation). Measurement should be performed at a fixed time, such as between 9:00-10:00 a.m.

Note: Gradual body weight loss (up to >10%) and frequent urination are indications of diabetes. EVENCARE G2 Blood Glucose is used for measuring blood glucose levels. Alternatively, Nutrisense CGM, Dexcom G6 can be used to measure and monitor blood glucose levels.

4. Mice with non-fasting blood glucose levels > 250 mg/dL measured on two consecutive days are considered diabetic.

Note: mice with blood glucose levels above 400 mg/dL are hyperglycemic and should receive 2 U of exogenous insulin before transplantation to facilitate post-surgery recovery.

Isolation of pancreatic islets

Timing: 3–3.5 h (for 12 mice) (for steps 5–8)

Timing: 15 min (for step 9)

We present a protocol for surgical procedures to accessing common bile duct, injection of liberase enzyme into pancreas, surgical removal and digestion of the pancreas, islet isolation using Ficoll gradients, staining islets with dithizone, and automatic counting. Collagenase NB1 and other gradients can be used[2] as alternative to liberase and Ficoll. Critical procedural steps are described and troubleshooting approaches for potential challenges are provided.

5. Remove the pancreas after perfusion
   a. Euthanize islet donor(s) mice either using CO_2 inhalation, cervical dislocation, or anesthetic agents.
   b. Disinfect the abdominal skin of mice by spraying it with 70% ethanol to maintain aseptic conditions throughout the isolation procedure.
   c. Create a 10-mm incision in the abdominal skin while preserving the integrity of the epidermis, dermis, and subcutaneous tissues in a linear fashion.
   d. Expose the abdominal cavity to make the pancreatic tissue visible by peeling the skin towards the inguinal area.
   e. Make approximately 1 cm cut in the thoracic cavity where the ribcage ends without damaging surrounding tissues.
   f. Locate the common bile duct all the way to the small intestine and clamp the proximal end of the bile duct close to the sphincter of Oddi called the ampulla of Vater in the duodenum using a bulldog clamp to avoid leakage of the Liberase solution into the intestine during perfusion (Figure 1A). Collagenase NB1 is a good alternative for Liberase.
   g. Using a 3-mL syringe with 31 or 27G needle (Figure 1B), depending on the bile duct thickness inject 3 mL Liberase solution into the common bile duct for perfusion (Figure 1C).

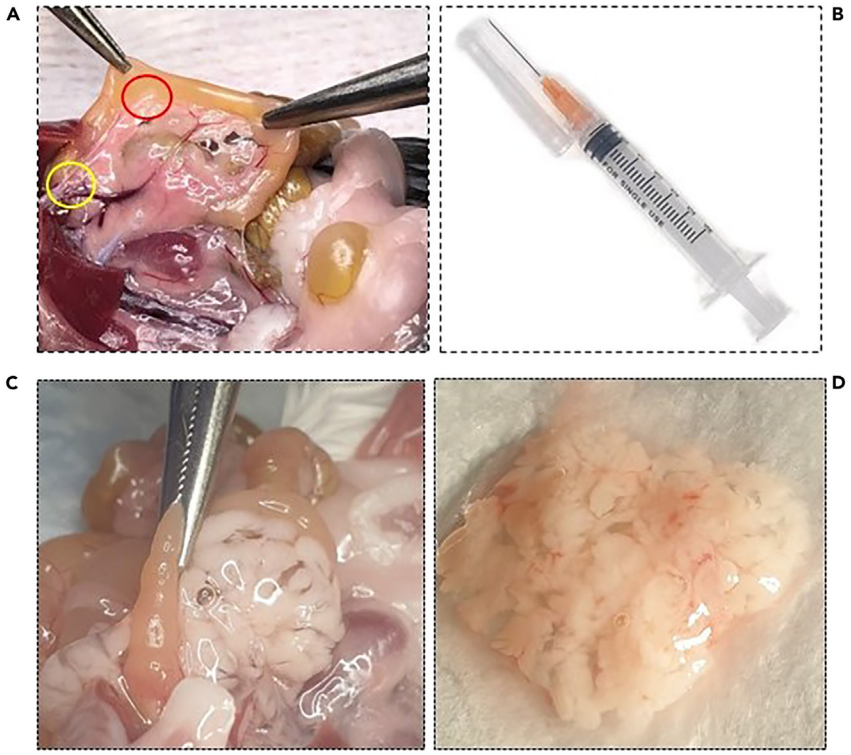

Figure 1. Pancreas perfusion and harvesting

(A) Locating the common bile duct (yellow circle) and the sphincter of Oddi (red circles) before injection.

(B) A 3-mL syringe with 31G needle loaded with 3 mL of collagenase solution.

(C) Clumping ampulla of Vater and injecting liberase solution.

(D) The perfused and inflated pancreas was separated from the surrounding tissue and placed in a Petri dish until further processing.

Note: To improve perfusion in the injection site of the common bile duct, carefully remove any fat and membranous tissues with the injection needle. This will expose the bile duct and make it visible under the microscope. Repetitive injections of the site can lead to damage of the bile duct and utmost care must be given to locate the specific lumen of the duct.

h. To remove the pancreas after assuring successful inflation, the clamp should be removed first and peel the pancreas from the surrounding connective tissue using fine forceps by starting from the head, which is nested at the curvature of the duodenum to prevent damaging arteries and veins associated with it.

i. Place the pancreas in a petri dish (Figure 1D) to remove the lymph nodes and fat tissue, if needed. Place the cleaned pancreas into a 50-mL Conical Falcon tube on ice. Two pancreases per 50-mL tube are recommended.

⚠ CRITICAL: To avoid excessive enzymatic digestion, harvest the pancreas within 1 h and keep on ice throughout. During the separation of pancreas, remove any fat tissue and control bleeding. Pancreas should remain intact after perfusion to avoid any leakage of the enzyme. Enzyme leakage can lead to insufficient digestion of the pancreatic tissue.

6. Digest the pancreas.

a. Incubate pancreas containing tubes at 37°C in a water bath for 10–12 min. Mice age should be considered to determine digestion time. The pancreas of older mice has relatively denser tissue and needs longer time for complete digestion.

⚠ CRITICAL: The enzymatic activity of liberase is time-dependent and varies from lot to lot and needs to be assessed beforehand. Use a plastic disposable Pasteur pipette (coated with coating buffer) to mix and homogenize the pancreatic tissue.

b. Keep tissue on ice after homogenization with the disposable pipette.
c. Stop enzymatic activity by adding 2 mL FBS into each tube. Mix it further using a Pasteur pipette and add 25 mL islet wash buffer.
d. Shake the 50-mL Falcon tube containing the digested pancreas for 30 s and spin at 150 × *g* for 1 min at 4°C.
e. Decant the supernatant and resuspend the pellets in 10–15 mL islet wash Buffer.
f. Filter the suspension through a 500-μm woven mesh filter. Rinse the tube with an additional 10 mL wash medium and pass through a 500-μm filter.
g. After resuspending, allow islets to settle down for 6–7 min at 18°C–25°C.
h. Remove supernatant by leaving the pellet in 5 mL solution to avoid loss of the islets during aspiration.
i. Wash 3–4 times, adjusting as needed based on the clarity of both the supernatant and the pellet.

⚠ CRITICAL: Islet size varies from strain to strain and age of mice.

7. Purify the islets.
   a. Coat a sterile 14-mL polypropylene round-bottom tube with a 30% coating buffer to prevent islets getting attached to the tube, which increases the yield, followed by transferring islet suspension.
   b. Rinse islets in the 50-mL falcon tube with an additional 2–3 mL islet wash buffer and transfer to a 14-mL polypropylene tube.

⚠ CRITICAL: Coat the 14-mL polypropylene round-bottom tube for two minutes with coating media.

   c. Spin down the islet suspension for 2 min (4 °C at 200 × *g*, 9 g acceleration, and deceleration) and decant the supernatant (SN) carefully without disrupting the islet pellet.
   d. Turn the biosafety cabinet light off and add 4 mL of 25% Ficoll solution to the islet pellet. Then, vortex to mix islets with Ficoll.

Note: Alternatively, a purification method without Ficoll can be used.[3] An efficient method of islet isolation from large animals using visipaque (iodixanol) density gradient has also been reported.[4]

   e. Overlay the islet solution with 2 mL of 23%, 2 mL of 20.5%, and 2 mL of 11% Ficoll sequentially by slowly pipetting the solutions to avoid mixing of formed layers.

Note: Mix each Ficoll solution before overlaying it onto the islet tube and change the pipet after each Ficoll solution.

   f. Spin down the solution at 750 × *g* for 20 min at 20°C. At this point, islets will settle at 11%–20.5% and 20.5%–23% interface.
   g. Prepare a volume of up to 12 mL wash media in a coated 50-mL Falcon tube and collect islets using a coated plastic pipette from each interface. One 50-mL tube is recommended for islets processed from not more than 6 pancreases.

Note: High purity islets are found at the 11%–20.5% interface, whereas low purity islets are found at the 20.5%–23% interface.

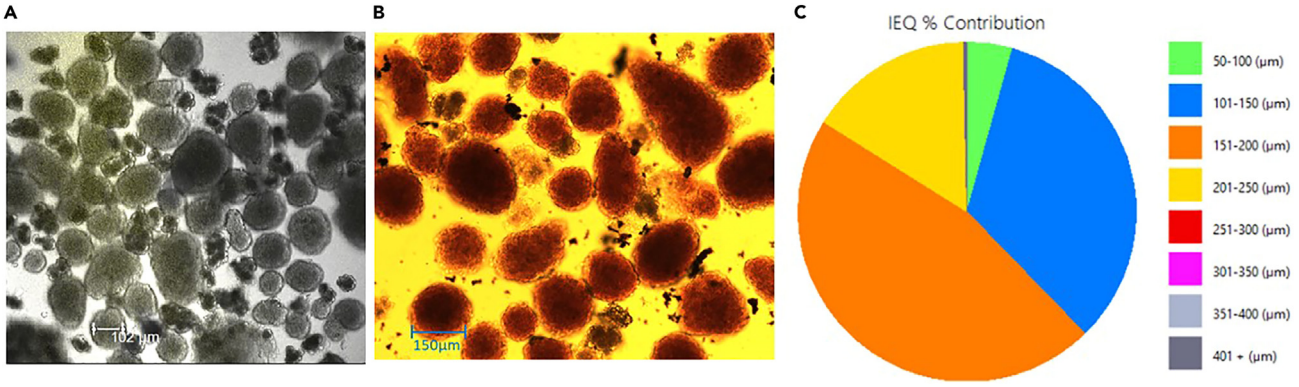

Figure 2. Islet purification and counting

(A) Representative isolated pancreatic islets from male BALB/cJ mice. The image shows purified intact islets under an inverted microscope.

(B) Representative pancreatic islets of BALB/cJ stained with iDTZ to differentiate between acinar and other tissue debris. Islets stain as crimson light red and exocrine acinar tissue stains as yellow.

(C) Representative manual count of BALB/cJ islets stained with iDTZ and distribution in percentages of IEQ based on size.

h. Mix islets well with the wash media and spin down at 250 × *g* for 1 min at 4°C.

i. Aspirate off the supernatant without touching the pellet and add 12 mL islet wash media to the pellet to purify the islets and repeat this step 3–4 times.

j. The last washing step should be done using 12 mL islet culture media.

8. Islet culture.

a. After the final wash, resuspend the islet pellet in 10 mL islet culture medium and transfer equal volume into a 100-mm culture Petri dish. Rinse the tube with 2 mL media to prevent loss of islets and transfer it to the Petri dish.

b. Check islet purity on unstained (Figure 2A) and stained islets with iDTZ (Figure 2B) by observing under light microscopy. iDTZ stained islets can be used for calculating total islets using different IEQ size distributions (Figure 2C).

⚠ CRITICAL: To prevent islet loss during processing and transferring to the culture Petri dish, it is a must to coat tubes and Pasteur pipettes with 30% coating buffer.

⚠ CRITICAL: Islets should be distributed evenly in the dish and approximately 300 islets can be cultured in 8–10 mL medium using a 60-mm Petri dish to prevent islet hypoxia.

9. Islet counting.

a. Automatic Cell Counting.

Automatic cell counting is conducted using Biorep Technologies Islet Cell Counter (ICC), which is an accurate and efficient way of calculating IEQ.[5] Manual islet counting can be used alternatively but ACC has an advantage as it addresses two main limitations of manual counting, speed, and inconsistency in counting.

i. Collected Islets from overnight culture into a coated 50-mL Falcon tube.

ii. After islets settle down, resuspend in 10 mL complete media for counting.

iii. Count islets according to the protocol given by Biorep automatic islet counting manual by mixing 100 µL islet suspension with 250 µL DTZ.

Note: After DTZ staining, islets can easily be identified from acinar cells and debris (Figure 3A). The machine segregates the total tissue mask from the selection mask by color, determines the boundary between islet cells (green) and surrounding non-stained particles or artifacts (blue), and gives an islet count on the region of interest (Figure 3B). Finally, the counter gives an automatic IEQ count distribution in percentage (Figure 3C).

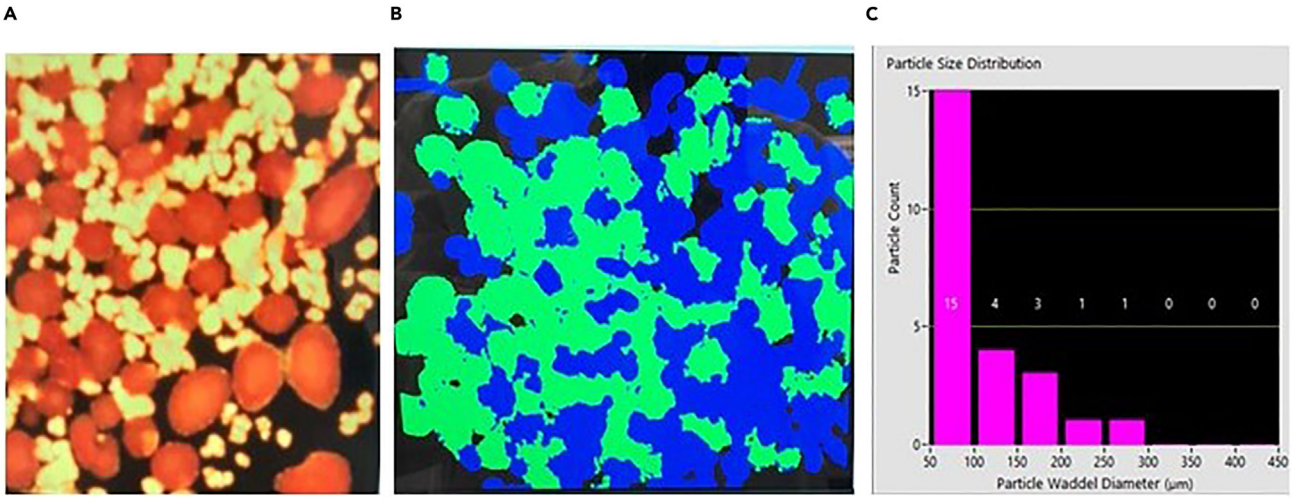

Figure 3. Procedures in automatic islet cell counting

(A) Islets stained with iDTZ for automatic islet cell counting.

(B) ICC software demarking islets (green) from artifacts (blue).

(C) Islet size distribution as percentages.

iv. Divide the Islet number per mouse/transplant and incubate islets in a coated 60 X 15-mm sterile Polystyrene culture dish in the complete media until transplantation.

v. Wash islets twice using PBS and collect using a coated 25G needle syringe for transplantation.

Parametrial fat pad islet transplantation (PFP)

Timing: 25 min per transplant

We present a protocol for transplantation of islets into PFP of diabetic syngeneic and allogeneic female recipients with key surgical procedures involving access to PFP, preparation of islets for injection, sealing PFP using thrombin and fibrinogen described, managing postoperative recovery of transplanted mice, and monitoring for blood glucose levels to assess engraftment. Critical procedural steps are described and troubleshooting approaches for anticipated challenges, such as peri/post-surgical complications, provided. The key surgical procedures are also provided in a supplementary video.

10. Anesthetize the mouse with the appropriate amount of avertin (125–130 mg/kg) or isoflurane (4% for induction and 2% for maintenance). Alternatively, ketamine (80–100 mg/kg) or xylazine (10–12.5 mg/kg) can be used to anesthetize mice.
11. Shave the fur from the lower quadrants (Figure 4A).
12. Place the mouse onto the surgical table, apply 70% ethanol onto the shaved area, and wipe the area (Figure 4B). In addition, topical application of betadine surgical scrub, and chlorhexidine scrub can be used as antiseptics.
13. Make a small midline incision following the peritoneal lining just at the lower.
    a. Quadrant (Methods video S1, 0.14 to 0.17).
14. Place a sterile drape around the surgical incision site.
15. Soak the gauze with sterile saline and pull out the fat pad onto the gauze using sterile forceps or Q-tip (Figure 4C).
16. Collect islets in the center of the 35 X 10-mm Petri dish by swirling the dish and then aspirate into 1-mL syringe under a stereomicroscope (Methods video S1, 0.03 to 0.06).

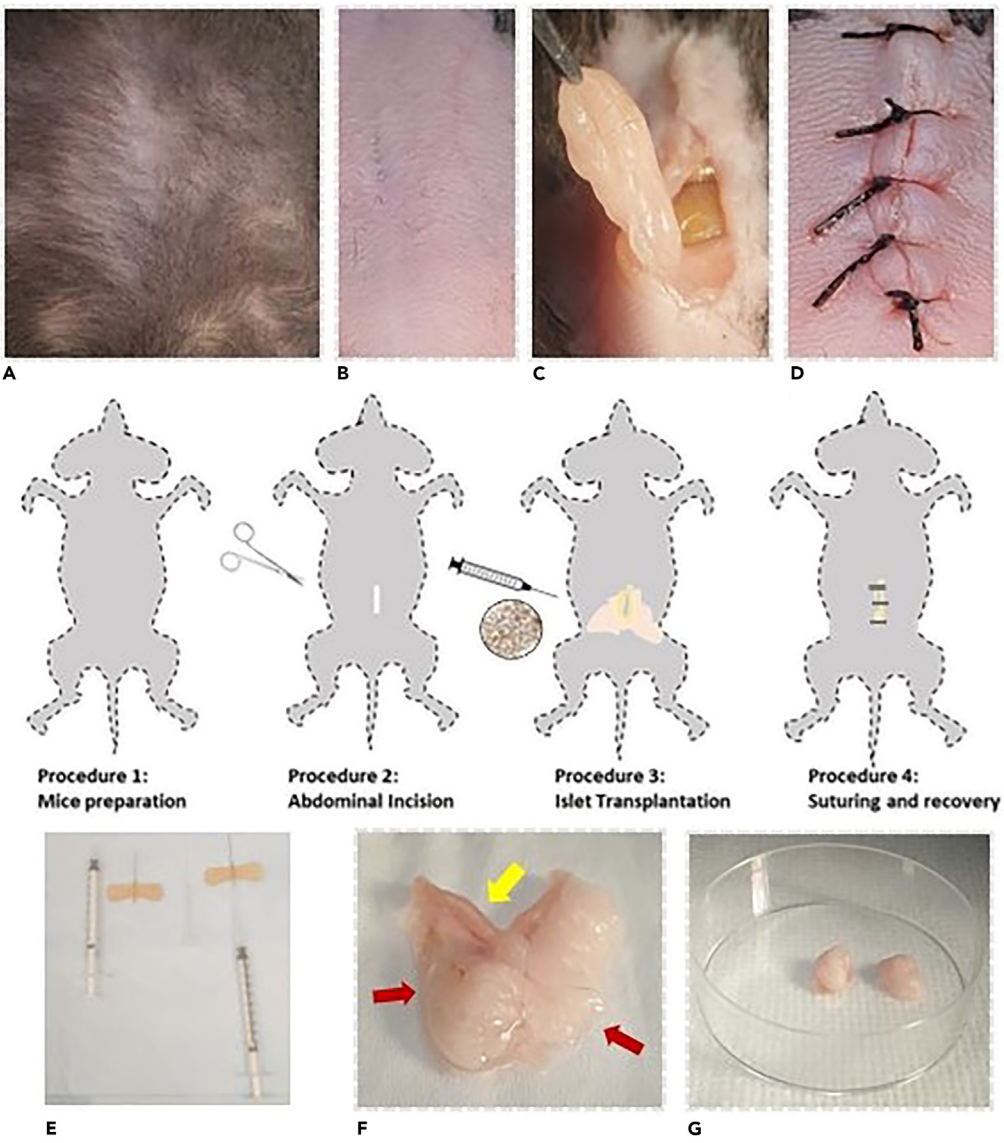

Figure 4. Surgical procedure for PFP transplantation

(A) Shaving abdominal hair and preparing an anesthetized mouse in a supine position.
(B) Applying hair removal cream to the shaved area to completely remove the hair.
(C) Making a surgical incision and pulling out the parametria fat pad tissue using delicate forceps for islet injection.
(D) Placing the tissue back to the abdominal cavity and suturing the area of incision.
(E) Assembling a 3-mL syringe with 25G butterfly needle for islet transplantation.
(F) Anatomical structure of the PFP (red arrows) with the uterine horns (yellow arrow).
(G) PFP harboring transplanted islets surgically removed after the experimental endpoint.

17. Invert the syringe to allow the islets to settle and attach a homemade needle (Figure 4E) to remove excess air bubbles and fluid, retaining approximately 30–50 μL solution. The syringe is then inverted with the 25G needle facing downwards and held in place to allow the islets to settle in the needle (Methods video S1, 0.09 to 0.12).

⚠ CRITICAL: Petri dish and syringe should be pre-washed with 30% FBS coating buffer to prevent islet adhesion. It is important to allow the islets to settle down in the needle to inject all islets into the tissue.

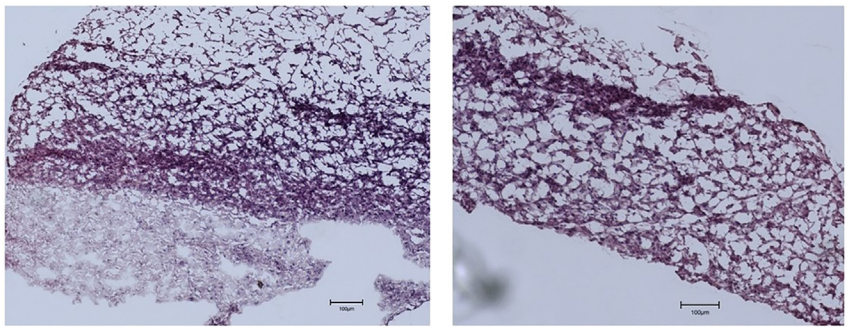

Figure 5. Hematoxylin and eosin staining of syngeneic islet harboring PFP tissue

18. Gently pull and hold the parametrial fat pad using a forceps and inject the islets into the sac (Methods video S1, 0.18 to 0.58).
19. Repeat the same steps for the other fat pad.
20. After injecting all islets, add 5–7 μL thrombin and the same volume of fibrinogen to the injection site.
21. Wait for 3–4 min to let the islets and thrombin/fibrinogen solution to settle inside the tissue.
22. Gently fold the tissue, reposition the fat pad within the abdominal cavity, and suture the muscle and then skin (Figure 4D). Following the surgery, administer analgesia and then place the mouse on a heating pad set at 37°C. Observe the mouse until it regains consciousness.
23. Provide postoperative analgesia to effectively deliver pain relief based on the time and dose specified.
24. Measure the first blood glucose levels on day 3 post-transplant and repeat the measurements twice a week for up to 30 days and then weekly thereafter.

⚠ CRITICAL: To avoid post-transplant bleeding and unnecessary post-surgical complications, do not pull too hard on the fat pad and surrounding tissues. Any pathological issue, such as necrosis, because of transplantation bleeding can be confirmed by postmortem examination on the parametria fat pad tissue (Figures 4F and 4G). Hematoxylin and eosin staining of OCT-embedded tissue (Figure 5) confirms the structural pattern of PFP tissue.

EXPECTED OUTCOMES

Mouse age, strain, and body weight are main factors that determine islet yield. Pancreas removal and processing impact isolated islet quantity and quality, which is assessed by purity and maintenance of a normal shape. Following isolation, islets tend to have a rough connective tissue capsule around the periphery due to exposure to mechanical and enzymatic stress during processing.[1,6,7] However, after overnight culture, the capsule becomes smoother once the damaged fibrous tissue sloughs off. The functional outcome of islets following transplantation is also impacted by islet size and index.[8]

The efficacy of parametria fat pad as a new site for islet transplantation into female mice was assessed using two syngeneic, C57BL/6-to-C57BL/6 and C57BL/6-to-F1 (C57BL/6xBALB/c), and two allogeneic models BALB/c-to-C57BL/6J and C57BL/6-to-NOD spontaneously diabetic mouse model. Transplantation of 450 IEQ in both chemically diabetic syngeneic models resulted in euglycemia long-term (Figures 6A and 6C) without a major health impact as assessed by body weight (Figures 6B and 6D). Transplantation of 1000 IEQ into diabetic allogeneic recipients also resulted in euglycemia within 3 days of transplantation in both allogeneic models, followed by acute rejection within 10–13 days (Figures 7A and 7C) without a major impact on the body weight (Figures 7B and 7D). Engraftment of islets into this new site in females was as effective as transplantation into the epididymal fat pad as an established site in male mice (Figure 8). These findings demonstrate that

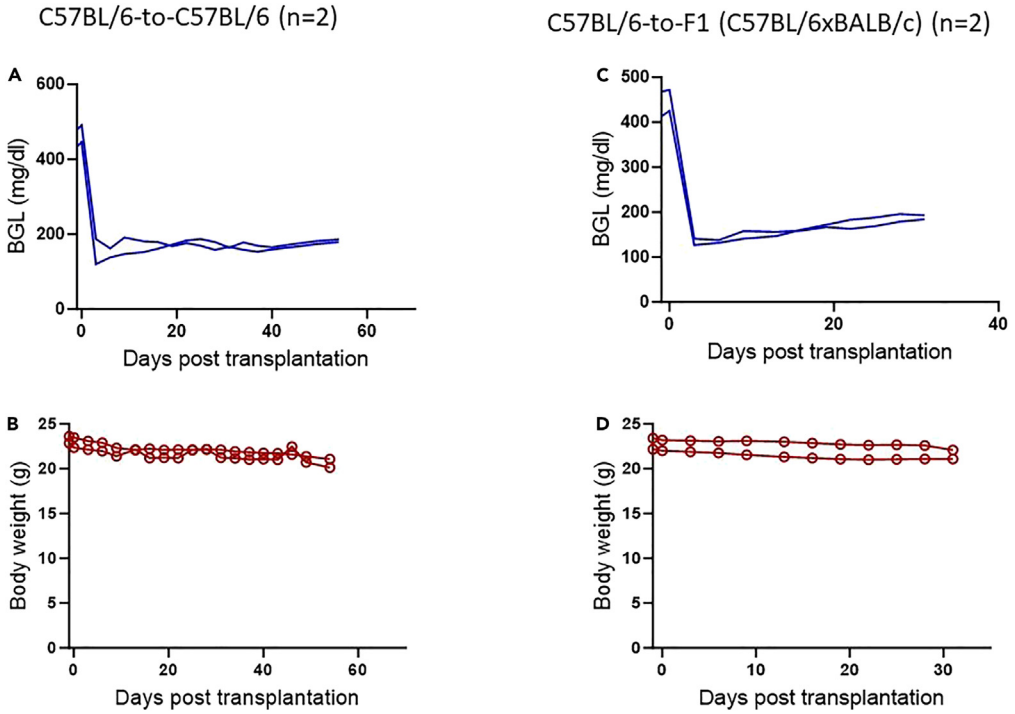

Figure 6. Engraftment and function of syngeneic islet transplanted into PFP of female recipients

(A) Blood glucose levels of C57BL/6 recipients of syngeneic C57BL/6 islets at various days post-transplantation.

(B) Body weight of mice in panel (A).

(C) Blood glucose levels of F1 (C57BL/6xBALB/c) recipients of C57BL/6 syngeneic islets at various days post-transplantation.

(D) Body weight of mice in panel (C).

the parametria fat pad in female mice is a suitable and equivalent site to the epididymal fat pad in males for islet transplantation.

Although various sites have been tested for islet transplantation in rodents, transplantation under the kidney capsule is an established and efficient site followed by intraportal transplantation. Furthermore, the efficacy of engraftment is much improved in the kidney capsule as compared to intra-portal transplantation.[9] This is most likely due to the occurrence of instant blood-mediated inflammatory reactions destroying a significant islet mass immediate post-transplantation, which is minimal in the case of kidney capsule.[10] Unlike the intraportal and kidney subcapsular as established sites in mice that are limited for the transplantation of large islet mass or combination with various biomaterial devices or encapsulation, the epididymal fat lacks such limitations. However, females lack the epididymal fat pad, and thus, establishing the parametria fat pad as an efficient alternative site for islet transplantation will facilitate studies focusing on sex bias as well as investigating the contribution of autoreactive responses to graft rejection in type 1 diabetes, which is significantly more prevalent in females. Furthermore, transplantation into the parametria fat pad is time efficient, less invasive, and lacks significant complications of bleeding as compared with transplantation into the epididymal fat pad (Figure 9).

LIMITATIONS

The quality of islets impacts the engraftment efficacy and depends on several key factors, including donor condition, the inherent characteristics of pancreatic tissue as well as the isolation and purification procedures employed. Mechanical and digestive stress and hypoxia during processing impact islet viability as well as their expression and secretion of various proinflammatory mediators

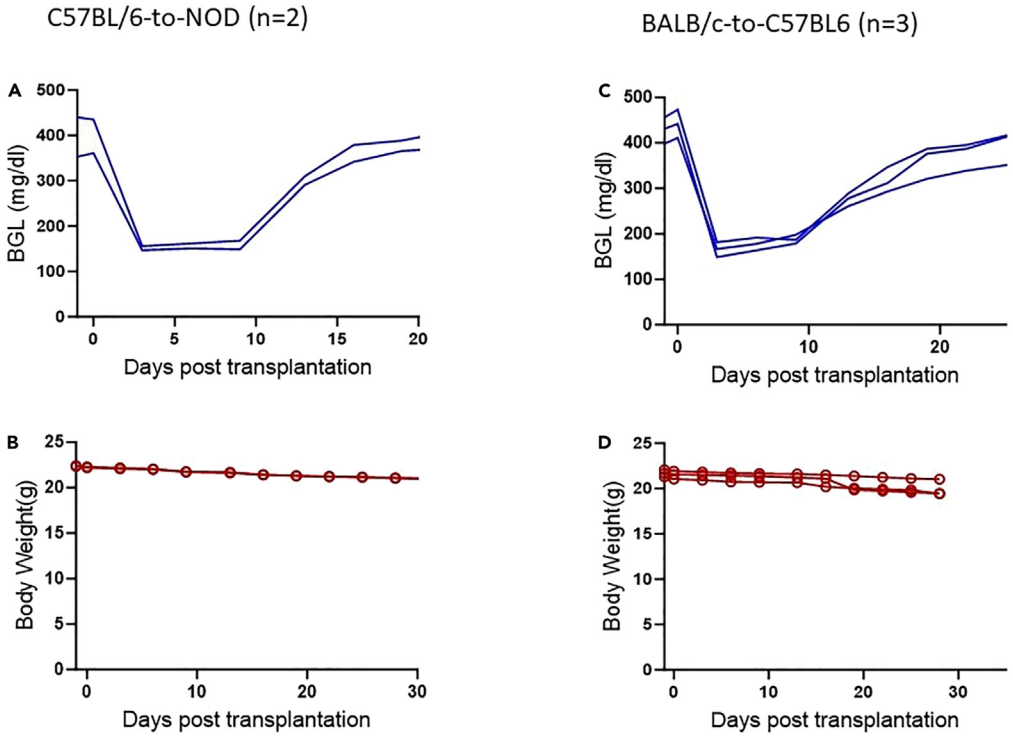

Figure 7. Engraftment and function of islets transplanted into PFP of allogeneic female recipients

(A) Blood glucose levels of spontaneously diabetic NOD recipients of allogeneic C57BL/6 islets at various days post-transplantation.

(B) Body weight of mice in panel (A).

(C) Blood glucose levels of chemically diabetic C57BL/6 recipients of allogeneic from BALB/cJ islets at various days post-transplantation.

(D) Body weight of mice in panel (C).

that influence engraftment.[6,11] Enzymes used to digest the pancreas may contain endotoxin that stimulates islet-resident macrophages to produce various proinflammatory cytokines impacting engraftment.[12] Thus, endotoxin-free enzyme should be used. Furthermore, enhancing processing skills to reduce the overall islet isolation time and avoiding excessive enzymatic digestion can enhance both the quality and engraftment function of islets.

TROUBLESHOOTING

Problem 1

Mice often develop intolerance to streptozotocin toxicity that causes mortality (Induction of diabetes by streptozotocin).

Potential solution

The induction of diabetes using streptozotocin varies from strain to strain. The potency of streptozotocin varies with mouse age, strain, and route of administration. Administration of 200 mg/kg of streptozotocin via tail vein is effective in inducing diabetes in C57BL/6 within 3 days. However, streptozotocin needs to be tested for diabetes induction without adverse effects for other strains. Streptozotocin solution should be prepared fresh, shielded from light to avoid degradation, and injected within 15 min of preparation for consistency of diabetes induction without adverse effects.

Problem 2

Poor pancreatic perfusion (Isolation of pancreatic islets).

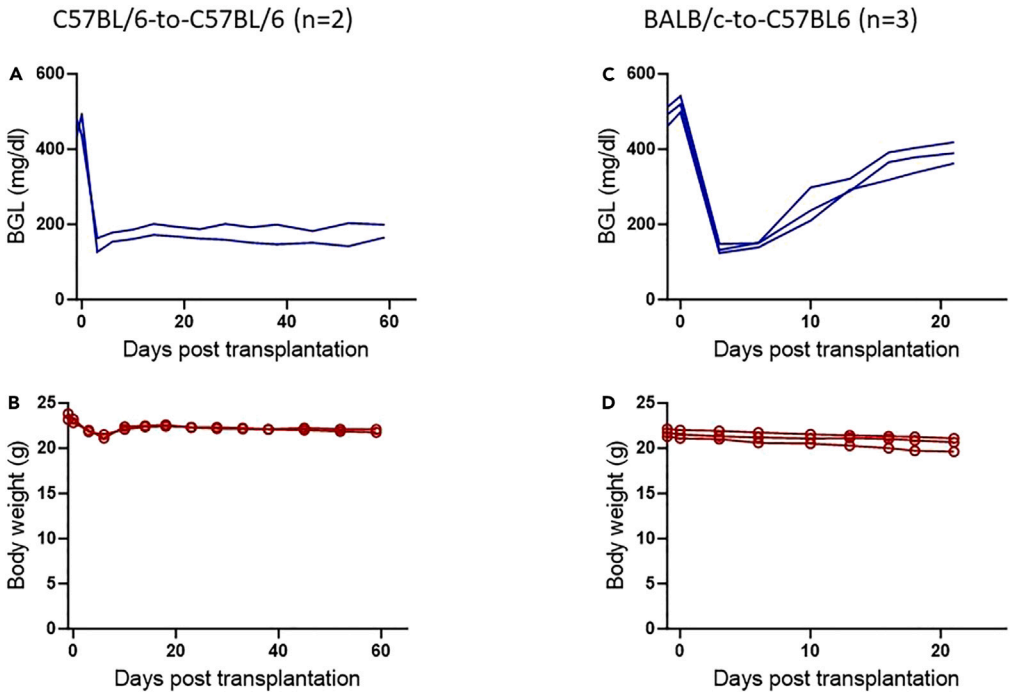

Figure 8. Engraftment and function of islets transplanted into Epididymal Fat Pad of male recipients

(A) Blood glucose levels of chemically diabetic C57BL/6 recipients of C57BL/6 syngeneic islets at various days post-transplantation.

(B) Body weight of mice in panel (A).

(C) Blood glucose levels of chemically diabetic C57BL/6 recipients of allogeneic BALB/cJ islets at various days post-transplantation.

(D) Body weight of mice in panel (C).

Potential solution

This results when the pancreatic duct is not appropriately clamped at the ampullary junction of the pancreatic duct and duodenum. In this context, efficient perfusion means when the entire digestive solution is entered into the pancreatic parenchymal tissue. Lack of proper clamping of the Ampulla of Vater causes the solution to diffuse into the large intestine. Identifying the architectural pattern of the ampulla of Vater at the intersection of the duodenum and distal part of the pancreatic duct helps a clear image for proper clamping. Partial clamping or the wrong position of clamping causes collagenase outflow, resulting in poor digestion and low islet yield and quality.

Problem 3

Damaging pancreatic duct during injection (Figures 1A and 1C).

Potential solution

It is a common problem to encounter a rupture of the duct during enzyme injection. The pancreatic duct is a delicate tubular tissue structure, and inserting a needle into the lumen might be challenging, particularly it the operator does not have adequate experience. Attempting to insert the needle more than twice may result in tube breakage, caused either by the needle itself or by droplets of enzymatic digestion at the insertion site. To prevent such issues, using an appropriate size of needle (32G or 30G) is recommended based on the size and structure of the pancreatic duct, which varies depending on mouse strains, age, and body weight. In addition, the duct is surrounded by adipose tissue, and removing this tissue before injection facilitates clear microscopic focusing that can contribute to the success of perfusion.

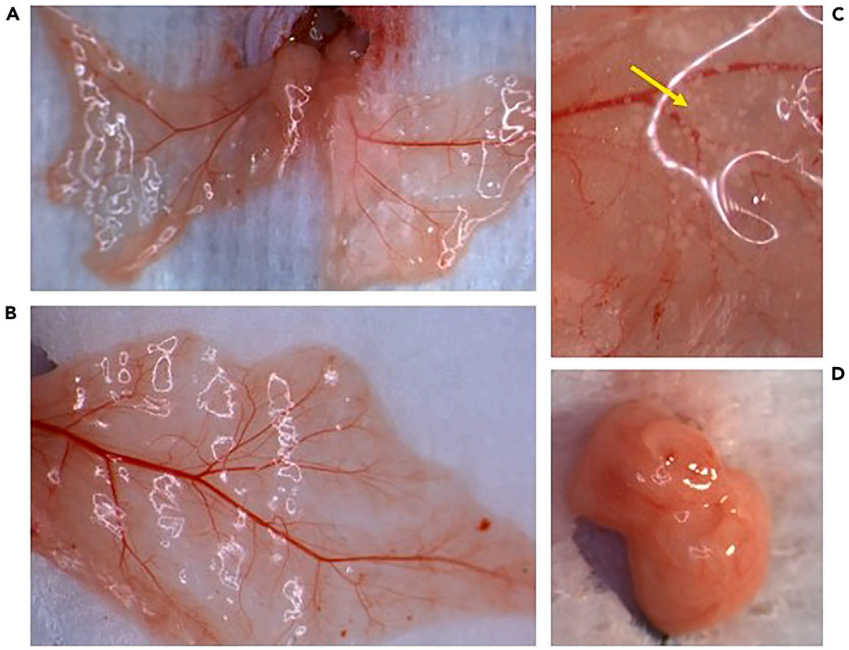

Figure 9. Epididymal fat pad transplantation of islets in male mice

(A) Both sides of the epididymal fat pad membrane (low magnification).

(B) One side of the epididymal fat pad membrane (high magnification).

(C) Islets transplanted in the epididymal fat pad membrane (indicated by yellow arrow).

(D) Folding the epididymal fat pad membrane.

Problem 4

Islet can be damaged due to excessive enzymatic digestion (Isolation of pancreatic islets).

Potential solution

Enzymatic over-digestion causes disruption of intact islet layers, affecting engraftment and function. Minimizing digestion time, usually between 10–15 min, depending on the pancreas size and age of mice, alleviates the problem. Under digestion can also cause low islet yield and poor quality.

Problem 5

Streptozotocin-treated mice (diabetic mice) (Induction of diabetes by streptozotocin) often exhibit poor body condition that is further impacted by the invasive surgical procedure, resulting in an incidence of mortality.

Potential solution

Although the frequency of death is very low, ultimate care must be given during the surgical procedure. For example, keeping the incision size small adequate for pulling out the parametria fat pad. During abdominal incision, a highly vasculature site needs to be considered to avoid bleeding. The time duration required from opening the abdominal cavity suturing should be minimal. From our experience, the procedure takes from 20–25 min.

Problem 6

During the surgical procedure, the fat pad can be damaged, and bleeding can occur which further affects the islet engraftment in the site (Figure 4. Surgical procedure for PFP transplantation).

Potential solution

To prevent such bleeding, the fat pad tissue should be handled gently with forceps and moderate pressure should be applied when the tissue is folded and repositioned back into the abdominal cavity.

Problem 7

Volume of PBS used to transplant islets (parametrial fat pad islet transplantation).

Potential solution

Depending on mice age and body condition, the size and thickness of the parametrial fad pad varies. As a result, the islet volume per transplant should not exceed 50 μL.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Haval Shirwan (haval.shirwan@health.missouri.edu).

Technical contact

Technical questions on executing this protocol should be directed to and will be answered by the technical contact, Mebrahtu G. Tedla (mtmpx@health.missouri.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate a unique code.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2023.102816.

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AUTHOR CONTRIBUTIONS

Methodology, M.G.T.; investigation, M.G.T.; writing – original draft, M.G.T.; writing – review and editing, M.G.T., N.W., E.S.Y., Y.W., and H.S.; funding acquisition, H.S., Y.W., and E.S.Y.; supervision, E.S.Y., H.S., and Y.W.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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