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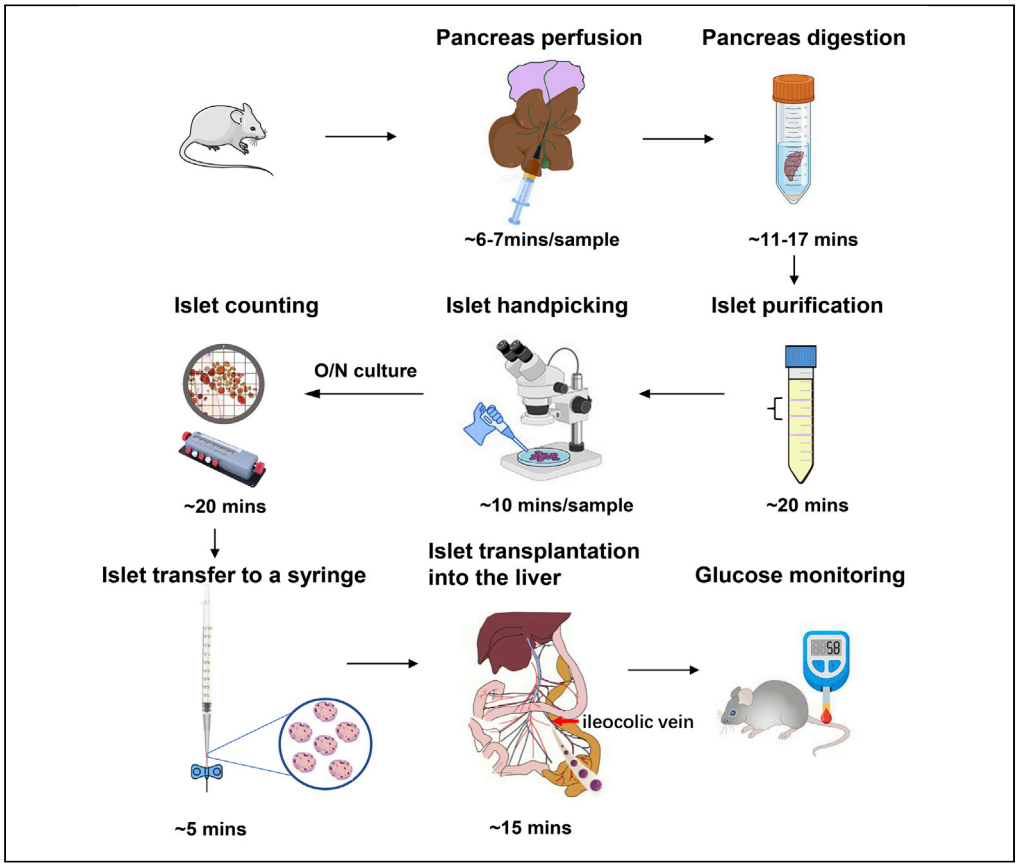
A modified surgical procedure using minimally invasive ileocolic vein perfusion in a mouse intrahepatic islet transplant model



Murine intrahepatic islet transplantation is a clinically relevant but technically challenging surgical procedure because of frequent lethal postoperative bleeding. Here, we describe a protocol for mouse pancreatic islet isolation, purification, and culture. Besides, we also describe a protocol for intrahepatic islet transplantation through the ileocolic vein. Intrahepatic islet transplantation through the ileocolic vein, as opposed to traditional islet perfusion via the main portal vein, has the advantage of improving recovery after surgery and may facilitate islet survival and function in preclinical settings.

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

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Highlights

Protocols describing the isolation and transplant of pancreatic islets in mice

High-quality islets and reverse hyperglycemia in STZ-induced diabetes in mice

Minimal incidence of post-infusion portal vein bleeding

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A modified surgical procedure using minimally invasive ileocolic vein perfusion in a mouse intrahepatic islet transplant model

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SUMMARY

Murine intrahepatic islet transplantation is a clinically relevant but technically challenging surgical procedure because of frequent lethal postoperative bleeding. Here, we describe a protocol for mouse pancreatic islet isolation, purification, and culture. Besides, we also describe a protocol for intrahepatic islet transplantation through the ileocolic vein. Intrahepatic islet transplantation through the ileocolic vein, as opposed to traditional islet perfusion via the main portal vein, has the advantage of improving recovery after surgery and may facilitate islet survival and function in preclinical settings.

For complete details on the use and execution of this protocol, please refer to Shrestha et al. (2020).

BEFORE YOU BEGIN

Institutional permission for animal experimentation.

All research and animal care procedures were in accordance with the policies of the NIH for guide, care and use of laboratory animals and protocols approved by the institutional animal care & use committee at the University of Missouri at Columbia. Mouse colonies were bred and maintained with standard mouse chow and water ad libitum.

Preparation for pancreatic islet isolation

Timing: 30 min

1. Set water bath temperature to 37°C.
2. Set centrifuge's temperature to 4°C.
3. Prepare islet wash solution.
4. Dissolve Liberase TL (0.18 mg/mL) in Ca^{2+} and Mg^{2+}-free Hank's balanced salt solution (HBSS).
5. Prepare a complete islet culture medium.
6. Prepare DTZ staining solution.
7. Prepare Ficoll solution.
8. Prepare clean surgical instruments.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Streptozotocin (STZ)	Sigma-Aldrich	Cat#S0130
0.9% Sodium Chloride Injection USP	B. Braun Medical Inc.	Cat#S8004-5264
HBSS, no calcium, no magnesium, no phenol red	Gibco	Cat#14175095
HBSS, calcium, magnesium, no phenol red	Gibco	Cat#14025092
Liberase™ TL Research Grade	Roche	Cat#5401020001
RPMI 1640 Medium, no glutamine	Gibco	Cat#21870076
Ficoll Type 400 DL	Sigma-Aldrich	Cat#F-9378
FBS	Gibco	Cat#16000044
L-glutamine	Gibco	Cat#25030-081
Penicillin-Streptomycin (10,000 U/mL- 10,000 μg/mL)	Gibco	Cat#15140122
2 ME (β-mercaptoethanol)	Sigma-Aldrich	Cat#M-7522,
DPBS	Sigma-Aldrich	Cat#D8537
Dithizone (DTZ)	Sigma-Aldrich	Cat#194832
HEPES (1 M)	Gibco	Cat#15630080
DMSO	ATCC	Cat#4-X
Methanol	Sigma-Aldrich	Cat#34860
Ammonium hydroxide solution	Sigma-Aldrich	Cat#338818
Experimental models: Organisms/strains		
BALB/cJ mice; male and female animals; 8–12-weeksold;	The Jackson Laboratory	Strain #000651
C57BL/6J mice; male and female animals; 8–12-weeksold;	The Jackson Laboratory	Strain #000664
Other		
Surgical scissors	Fine Science Tools	Cat#9140012
Small curved surgical scissors	Electron Microscopy Sciences	Cat#729355
1.5-inch bulldog clamp	Fine Science Tools	Cat#1805135
Ultra-Fine Point Tweezers	Fisherbrand	Cat#12000122
30G × 1/2″ needle	EXELINT International	Cat#26437
3 mL syringe	BD	Cat#309657
50 mL tubes	Fisherbrand	Cat#05-539-13
100 mm petri dish	Fisherbrand	Cat#FB0875712
60 mm petri dish	Fisherbrand	Cat#FB0875713A
Falcon 17 × 100 mm, 14 mL round polypropylene bottom test tubes	Life Sciences	Cat#352059
7×–45× Stereo Zoom Inspection Industrial Microscope	Amscope	Cat#SM-1BN
Inverted cell culture microscope	Olympus	Cat#CKX53
Nylon Mesh Filters, 500 μm,	Tisch Scientific	Cat#ME17264
General-purpose water bath	Fisher Scientific	Cat#FSGPD20
Centrifuges	Thermo Scientific	Cat# Sorvall X Pro Series
Portable balances	OHAUS	Cat# SCOUT™ SPX
CO2 incubator	Thermo Scientific	Cat# Forma Series II HEPA Class 100
Surflo winged infusion set	Terumo	Cat# SV*25BLS
Gel-Loading Tips, 1–200 μL	Fisherbrand	Cat#02-707-81
1 mL syringe	BD	Cat#309623
Absorbable Gelatin Sponge	Ethicon	Cat#1972
7×–90× Binocular Stereo Boom Microscope + Ring Light	Amscope	Cat#SM-4BY-FRL
EVENCARE G2 Blood Glucose Meter	Medline	Cat#MPH1540NV
EVENCARE G2 glucose stripes	Medline	Cat#MPH1550

MATERIALS AND EQUIPMENT

Islet wash solution

Reagent	Final concentration	Amount
FBS	1%	5 mL
HBSS with Ca^{2+} and Mg^{2+}	N/A	485 mL

(*Continued on next page*)

Continued		
Reagent	Final concentration	Amount
HEPES (1 M)	20 mM	10 mL
Total		500 mL

0.18 mg/mL Liberase LT solution

- Dissolve 5 mg Liberase TL in 27.7 mL Ca^{2+} and Mg^{2+}-free HBSS.

Note: The solution is sufficient for 9 mice and should be prepared fresh, kept on ice, and used within 1 h.

Complete islet culture medium		
Reagent	Final concentration	Amount
FBS	10%	5 mL
L-Glutamine (200 mM)	2 mM	0.5 mL
Pen/Strep (10,000 U/mL and 10,000 μg/mL)	100 U/mL and 100 μg/mL	0.5 mL
2-Me (50 mM)	50 μM	0.5 μL
RPMI-1640	N/A	43.5 mL
Total	N/A	50 mL

Note: Prepare all solutions fresh, keep on ice, and use within one day.

iDTZ stock solution (8 mg/mL)

iDTZ solution		
Reagent	Final concentration (v/v)	Amount
DMSO	62%	6.2 mL
Methanol	37.5%	3.75 mL
Ammonium hydroxide solution	0.5%	50 μL
Total		10 mL

- Dissolve 80 mg Dithizone in 10 mL iDTZ solution and vortex for 30 seconds.
- Filter the dissolved solution using a 0.45 μm nylon filter.
- Store at −20°C for 6 months.

Note: The iDTZ solution should not be exposed to light and is used to stain islets at a 1:10 or a greater dilution.

Ficoll solution preparation

- Dissolve 25 g Ficoll in 75 mL HBSS with Ca^{2+} and Mg^{2+} to obtain 25% stock solution.

Note: Dissolve Ficoll completely by stirring using magnetic mixer overnight, sterilize by passing through a 0.45 μm nylon filter, and keep at 4°C until use.

- Prepare following Ficoll solutions:
- 23%: 46 mL of the 25% Ficoll + 4 mL HBSS.
- 20.5%: 41 mL of the 25% Ficoll + 9 mL HBSS.

- 11%: 22 mL of the 25% Ficoll + 28 mL HBSS.

Note: Keep the solutions in dark at 4°C until use.

STEP-BY-STEP METHOD DETAILS

Induction of diabetes by streptozotocin

Timing: 30 min

1. On day -4, measure the body weight of C57BL/6J mice (7–10-week-old) using a digital scale.
2. Prepare 20 mg/mL solution of streptozotocin (STZ) in 0.9% saline.

Note: STZ-saline should be prepared fresh just prior to injection to avoid STZ degradation.

3. Inject STZ solution into a mouse via tail vein at dose of 200 mg/kg using a 27G needle.

Note: Prior to injection, warm animals for 5–10 min to dilate the veins to facilitate injection.

4. Measure the blood glucose level (BGL) and body weight on day -1 post STZ injection. Measure BGL at fixed time frame, such as between 9:00–10:00 a.m.

Note: Weight loss and polyuria are hallmarks of diabetes and may help to predict the onset of diabetes. In general, excessive weight loss (>10% of initial body weight within 4 days) is an indication of STZ toxicity, which is expected.

5. Mice with non-fasting blood glucose >300 mg/dl on day -1 and day 0 with respect to islet transplantation are considered diabetic and used for transplantation.

Note: Mice with non-fasting blood glucose levels greater than 400 mg/dl on days -1 and 0 can be given 2 U of exogenous insulin on days 0–3 post transplantation to alleviate diabetic stress.

Note: We use EVENCARE G2 Blood Glucose Meter system for blood glucose monitoring. Other brands also can be used.

Isolation of pancreatic islets

Timing: 2.5–3 h (for 4 mice) for steps 6–9

Timing: 20 min for step 10

6. Pancreas perfusion and removal.
   a. Euthanize islet donor using short CO_2 inhalation followed by secondary physical method of cervical dislocation or anesthetic reagents to confirm euthanasia.
   b. Clean the mouse dorsal and ventral external surfaces area with 70% alcohol to prevent fur contamination during dissection.
   c. Lift the lower abdominal skin with forceps and make a small (10 mm) cut through the epidermis and dermis leaving underlying membrane intact with scissors.
   d. Using forceps, carefully peel the skin away from the abdominal cavity, exposing the entire abdominal cavity to the extremities.
   e. To reveal the abdominal cavity, make a horizontal cut (~1 cm) where the diaphragm/rib cage ends without damaging the liver.
   f. Then make vertical cut toward the limb on both sides.
   g. Open the abdominal cavity and spray viscera with saline solution.

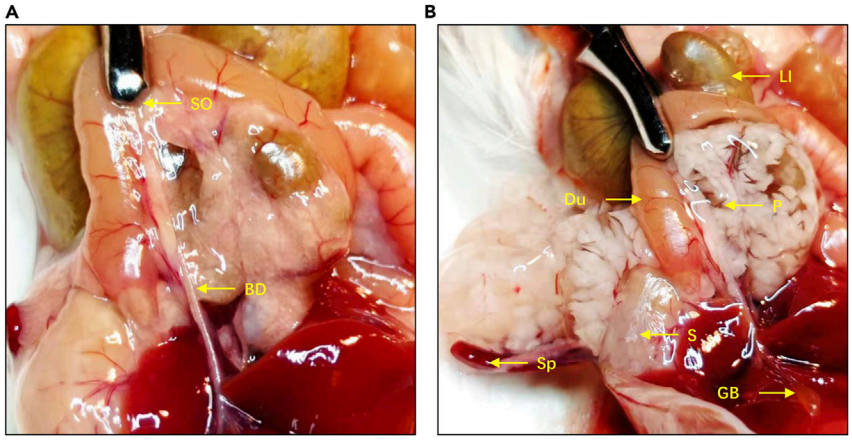

Figure 1. Pancreas perfusion

(A) Close-up image of the bile duct. The abdominal cavity showing the pancreas before inflation.

(B) The inflated pancreas after collagenase injection through the bile duct. BD, bile duct; GB, gallbladder; P, pancreas; S, stomach; Sp, spleen; LI, large intestine; Du, duodenum; SO, sphincter of Oddi.

h. Under the dissecting microscope, locate the common bile duct until it reaches the small intestine (Figure 1A). Clamp the bile duct close to the sphincter of Oddi with a bulldog clamp to prevent the collagenase solution from entering the intestine during pancreas inflation.

i. Inflate the pancreas with 2.5–3 mL of cold Liberase solution using 3 mL syringe with 31G needle. Inflation is complete when the entire pancreas (including the tail section adjacent to the spleen) became engorged and appears gelatinous (Figure 1B).

Note: Other collagenases can also be used to isolate islets from the pancreas. Collagenase V and collagenase P, for example, but the time required for digestion varies depending on the enzyme and animal source. Prior to conducting batch experiments, enzyme should be titrated to determine the optimal concentration and incubation time.

j. First remove clamp then carefully separate the pancreas from the surrounding tissues and place it in a petri dish to remove the lymph nodes and fat tissue, if needed, before placing it in a 50 mL Conical Falcon tube on ice.

⚠ CRITICAL: Try to puncture pancreas as close to the hepatic hilum as possible. In case of failure, you can continue to re-puncture distally. Try to clamp the sphincter of Oddi. If the clamp is incomplete, the perfusate will enter the small intestine; if too much is clamped, the pancreas will be under perfused.

⚠ CRITICAL: Pancreas harvest must be completed within 1 h to prevent excessive enzymatic digestion.

7. Pancreas digestion.

a. Transfer the tubes to a 37°C water bath and incubate for 11–12 min without agitation.

⚠ CRITICAL: Optimization of incubation time may be necessary as Liberase activity may vary from batch to batch. Examine islets under a microscope to assess if they are under- or over-digested.

b. Use a disposable 10 mL pipette to pipet up and down the pancreatic tissue until becomes homogeneous.

c. Stop the enzymatic reaction first by adding 2 mL of FBS, after thoroughly mixing to achieve a homogenous suspension, then add 20 mL cold wash medium. Followed by closing the 50 mL falcon tube cap and shaking the tube until a fine sand-like consistency is obtained.
d. Spin at 150 × *g* for 1 min at 4°C with 9 g acceleration and deceleration.
e. Decant the supernatant and resuspend the pellet in 10 mL wash medium using a pipet.
f. Filter the suspension through a 500 μm woven mesh filter. Rinse the tube with an additional 10 mL of wash medium and pass through a 500 μm filter.
g. Let the cells settle for 3 min at 18°C–25°C. Aspirate the supernatant off with a 10 mL pipette until only 5 mL of solution remains, being careful not to disturb the pellet. This wash step should be repeated three times.

8. Islet purification.
a. Transfer the islet suspension in a STERILE, 14 mL polypropylene round-bottom tube, which is coated with 30% FBS solution (2–3 pancreases/tube).
b. Rinse the 50 mL falcon tube with 2–3 mL additional wash medium and transfer it to a 14 mL polypropylene tube, maintaining the final volume at 12 mL.

⚠ CRITICAL: Coat 14 mL polypropylene round-bottom tube with coating media (Coating media is 30% FBS -wash media).

c. Spin for 2 min at 4°C at 200 × *g* with 9 g acceleration and deceleration. Decant the supernatant (SN) and then drain the access of SN by tilting the tube at a very low angle to remove remaining SN.

Note: At this point, the islet pellet will be loose; keep an eye on it while removing the remaining wash media to avoid a change in Ficoll density.

d. Turn off the light, add 4 mL of 25% Ficoll over the pellet by using the low-speed pipetting technique, and then vortex the tubes to mix well the islet pellet with Ficoll. Overlay 2 mL of 23%, 20.5%, and 11% Ficoll sequentially.
e. Change the centrifuge settings to minimal acceleration with no brake and spin the tubes at 750 × *g* for 20 min. The islets will migrate to the 11%–20.5% and 20.5%–23% interface (Figure 2A). Use a disposable plastic pipette to transfer the islets into two FBS-coated 15 mL Falcon tubes with 5 mL of wash media and 1 mL of FBS. Transfer each interface to a different 15 mL tube.

Note: Islets with higher purity are at the 11%–20.5% interface, while those with lower purity are at the 20.5%–23% interface.

Alternatives: While Ficoll-400 is the most widely used density gradient medium, other solutions such as Histopaque-1077 are also frequently used for islet separation (McCall et al., 2011) based on the principle that islets are separated by centrifugal force due to their distinct density from the exocrine tissue.

f. Bring the volume to 12 mL with wash media and spin at 250 × *g* for 1 min at 4°C.
g. Aspirate off the supernatant and add 12 mL of complete medium to wash the islets. Repeat this wash step for a total of three times.

9. Islet culture.
a. Resuspended islet pellet in 10 mL medium and transfer it to a 100 mm petri dish. Rinse the tube with an additional 5 mL of complete medium and transfer to the petri dish.
b. Carefully handpick islets with a 10 μL pipette tip using a stereo microscope with bottom lights (Figures 2B and 2C) and transfer them to a 15 mL tube with 2 mL of complete medium.

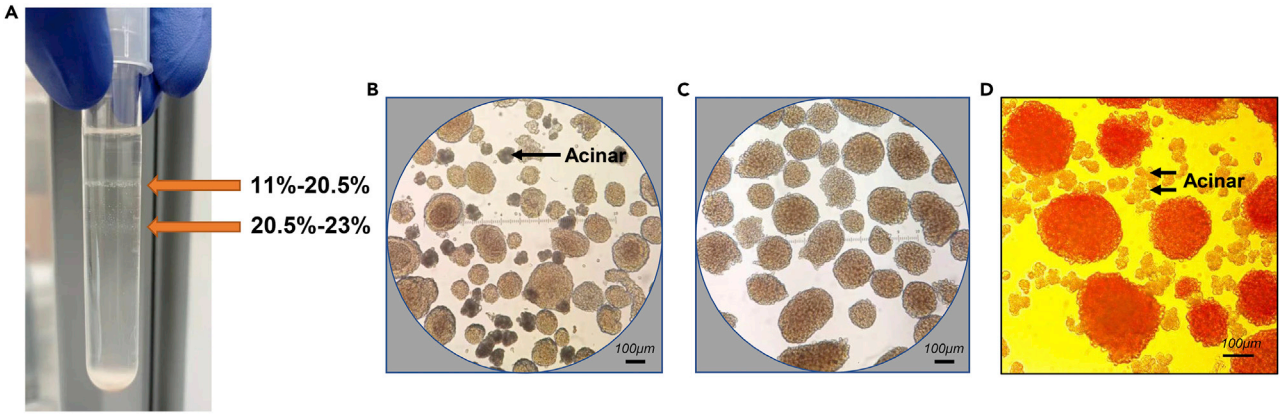

Figure 2. Islet purification and handpicking

(A) Pancreatic islets were purified by discontinuous density centrifugation with Ficoll solution. Majority of islets locate to the 11%–20.5% interface, while less pure islets are at the 20.5%–23% interface.

(B) Representative image of isolated islets from 8-week-old female BALB/cJ mouse before handpicking. Arrow shows exocrine acinar cells in the islet preparation.

(C) Almost 100% pure islets after handpicking. Images were taken under an inverted microscope at 10 × magnification.

(D) Islets can be easily distinguished from exocrine tissue by DTZ that stains islets as crimson red.

c. Once all the islets are collected, transfer islets to a sterile 60 mm petri dish and incubate overnight (14–16 h) at 37°C and 5% CO_2.

⚠ CRITICAL: To avoid islet loss during transferring, ensure all the tubes, pipet, and dishes are coated with 30% FBS coating buffer. Petri dishes are not tissue culture-treated to maintain islets in suspension.

⚠ CRITICAL: To avoid islet hypoxia, do not culture more than 300 islets in a 60 mm petri dish with the 8–10 mL medium. Distribute islets evenly throughout the dish to avoid aggregation.

10. Islet counting.
    a. Samples are taken and stained with DTZ. Islets are measured and counted under the microscope. Islets are calculated as Islet Equivalent (IEQ) per the following table.

Islet diameter rang (μm)	Actual islets present (AI)	IEQ conversion factor	IEQ
50–100		× 0.167 =	
101–150		× 0.648 =	
151–200		× 1.685 =	
201–250		× 3.500 =	
251–300		× 6.315 =	
301–350		× 10.352 =	
>350		× 15.833 =	
	Islets in Sample (ΣAI) =	IEQ in Sample (ΣIEQ) =	
		Dilution Factor: (mL total volume / μL sample volume) × 1000 =	
		Total AI: ΣAI × Dilution Factor =	
		Total IEQ: ΣIEQ × Dilution Factor =	

Note: Add 1–2 drops of iDTZ stock solution into 100 μL of islet solution. Allow islet staining with DTZ 1–2 min at 18°C–25°C. Islets can be distinguished from exocrine tissue due to scarlet staining (Figure 2D).

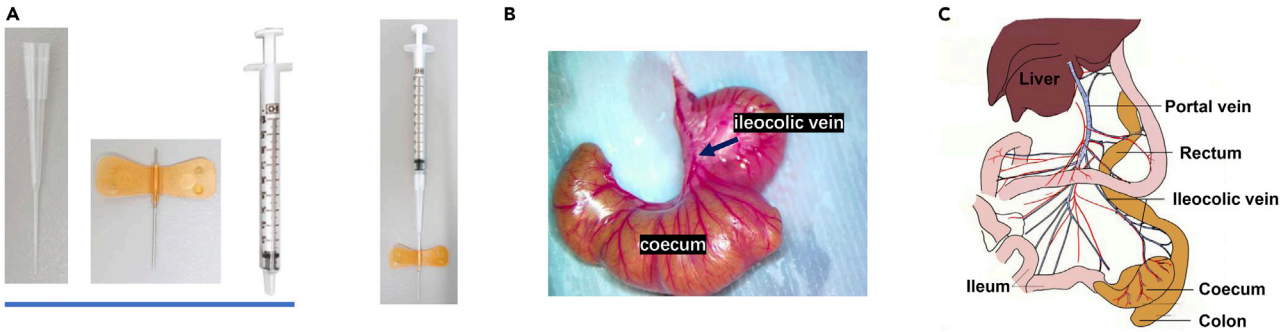

Figure 3. Preparation and perfusion of islets into the liver by the ileocolic vein, which is a terminal branch of portal vein

(A) Islet perfusion set built in-house, including loading tip, butterfly needle, and 1 mL syringe. This instrument ensures that islets settle down to the needle, allowing for a complete islet injection into the portal vein.

(B) The ileocolic vein can be easily exposed without disturbing other abdominal organs.

(C) Schematic diagram of the anatomy of the portal vein system in the mouse.

Note: This IEQ counting formula is also used in the clinic. It is more accurate to apply IEQ for islet transplantation due to the different sizes of islets that facilitates standardization of islet transplantation to accomplish comparison of data among different laboratories.

Islet intrahepatic transplantation

Timing: 30 min per transplantation

11. Transfer viable islets by excluding those with darker core is greater than 50% of the total area of the islet (Figure 4) (damaged due to hypoxia) to 15 mL tubes and wash with PBS 2 times.
12. Transfer islets into the 6 cm petri dish containing PBS.
13. Islets are collected in the center of the dish by swirling the petri dish and aspirated into the 1 mL syringe under a stereomicroscope (Methods video S1: Islet intrahepatic transplantation, related to step 13).
14. Invert the syringe to allow the islets to settle and attach a homemade needle (Figure 3A) to remove excess air bubbles and fluid, retaining approximately 200–300 μL solution. The syringe is then inverted with the needle facing downwards and held in place to allow the islets to settle near the needle (Methods video S1: Islet intrahepatic transplantation, related to step 14).

⚠ 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 bloodstream without a loss. This step is especially critical when marginal islet numbers (such as 100–200 IEQ/Tx) are used for transplantation.

15. The recipient is anesthetized, shaved, and the abdomen is sterilized with 70% ethanol. A midline 1–1.5-cm abdominal incision is made to localize the ileocecal region and fully expose the ileocolic veins (Figures 3B and 3C) and (Methods video S1: Islet intrahepatic transplantation, related to step 15).
16. Pierce into the Ileocolic vein with 25G needle distally and infuse the islets slowly. Then press the hemostatic sponge against the puncture point, retract the needle, and compress to stop bleeding using absorbable gelatin sponge for 2–3 min, (Methods video S1: Islet intrahepatic transplantation, related to step 16).

Note: The injection volume should not exceed 300 μL as excess fluid may cause circulatory overload in the mouse.

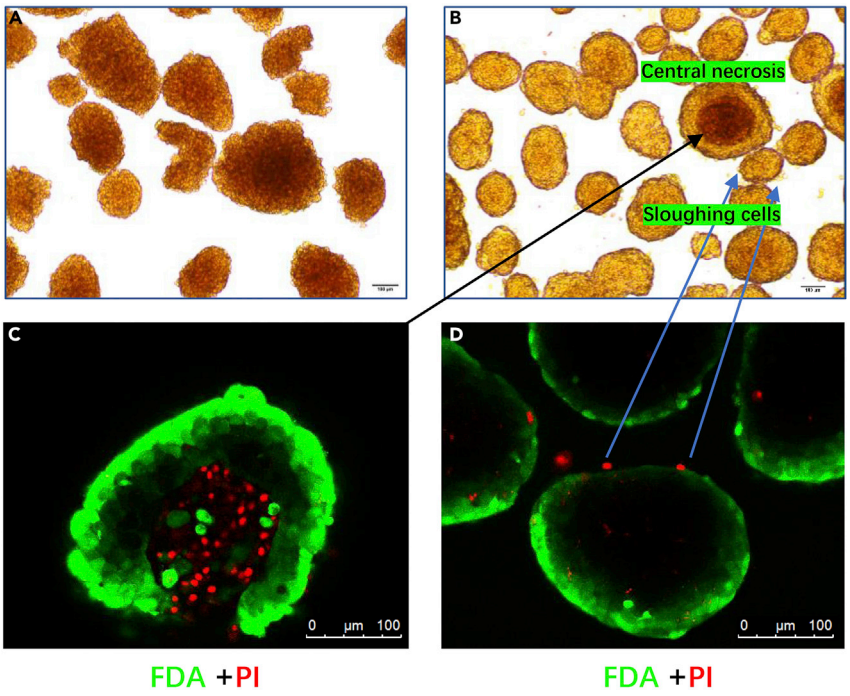

Figure 4. Representative images of purified islets

(A) The surface of freshly isolated islets is rather rough, plausibly due to the outer cell layer disruption during the enzymatic isolation procedure.

(B–D) Overnight cultured islets have smooth peripheral surface arising from the recovery of islets from isolation-induced stress. Occasionally, dark central region in larger islets (C) and sloughing of cells (D) from the islet periphery are visible. These cells stain positive for PI that marks nonviable cells. Images were taken by Leica SP5 confocal microscope at 20 × magnification.

17. Carefully incorporate the intestine back into the abdominal cavity and close the abdomen. Post-surgery, provide analgesia, place the mouse on a heating pad at 37°C and observe until the mouse is awake and active (Methods video S1: Islet intrahepatic transplantation, related to step 17). Provide postoperative analgesia to effectively deliver pain relief based on the time and dose specified.

⚠ CRITICAL: To avoid post-transplant bleeding, do not pull too hard on the injected vessel.

18. Measure the blood glucose levels at least twice a week for up to 30 days and then weekly afterward.

EXPECTED OUTCOMES

We routinely obtain 150–250 IEQ per BALB/cJ mouse and 100–200 islets per C57BL/6J using the protocol described above. However, the number and size of islets are variable, depending on the animal strain and age. Freshly isolated islets typically have a rough peripheral border, which results from the stress during isolation (Figure 4A). After overnight culture, the healthy islets recover and show smooth membrane due to sloughed off dead cells (Figures 4B and 4D). For the intrahepatic islet transplantation model, we found that relatively small islets are better for transplantation as they are less prone to hypoxia, whereas large islets are more susceptible to central necrosis developing during culturing (Figure 4C). In addition, the small islets reduce the chance of obstructing the major branches of the portal vein, thus facilitating successful engraftment within the hepatic lobules (Figure 5). In our experiments, 80% of the islets obtained were measured between 100 µm and

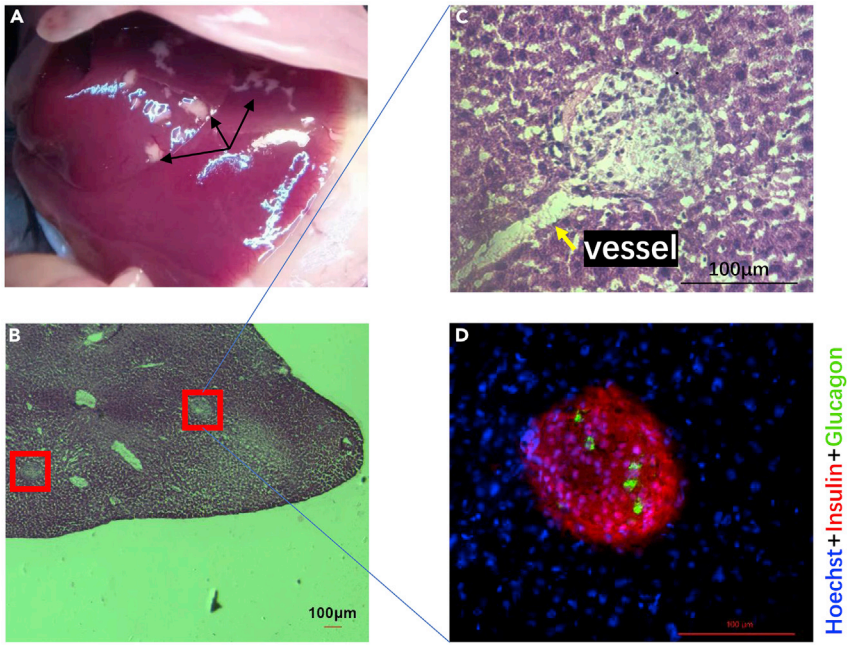

Figure 5. Representative pictures of transplanted pancreatic islets into liver

(A) After islet transplantation, white areas appear on the surface of the liver where the islets are present. The red blood vessel adjacent to the islet is most likely due to local ischemia induced by islet thrombosis, which blocks small blood vessels. This is a transient occurrence that will go away over time.

(B) The islets are engrafted within the hepatic blood sinusoids. H&E staining at 4 × magnification.

(C and D) H&E (C) and immunofluorescence (D) staining showing good islet structure. Images were taken by Olympus inverted microscope and Leica SP5 confocal microscope at 20 × magnification.

250 μm with viability of approximately 95%–100%. Compared to conventional portal vein trunk transplantation (Khatri et al., 2018), the application of terminal branches of the portal vein (ileocolic veins) to import islets into the liver is simpler and more convenient with less surgical stress to the recipient and a lower chance of bleeding. (Methods video S1: Islet intrahepatic transplantation, step 16).

In C57BL/6J syngeneic transplantation, 400 IEQ reversed diabetes and survived indefinitely (Figures 6A and 6B). In BALB/CJ-to-C57BL/6J allogeneic transplantation, 700 IEQ islets were required to reverse diabetes, and rejection was observed between 6 to 21 days (survival time=10.8 ± 5.3 days) (Figures 6C and 6D). This route of transplantation mimics clinical setting and provides a less invasive means of intraportal transplantation into mice with minimal bleeding complications to study immune mechanisms of rejection and assess therapeutic immune interventions (Cantarelli et al., 2013; Merani et al., 2008).

LIMITATIONS

The process of islet isolation triggers a series of stressful events, leading to cell death and production of various proinflammatory cytokines, chemokines, and danger molecules that impacts islet viability and function in vitro as well as in vivo following transplantation. With intrahepatic islet transplantation via the portal vein, postoperative abdominal bleeding remains the principal cause of early death in mice, which is related to both inadequate hemostasis and portal hypertension due to thrombosis from islet obstruction (Kawahara et al., 2011).

Another major issue with intraportal islet transplantation is early islet loss, which can be caused by several factors, including ischemia/reperfusion, an instant blood-mediated inflammatory reaction (Bennet et al., 2000), inherent hepatic immune cell microenvironment (Delaune et al., 2017),

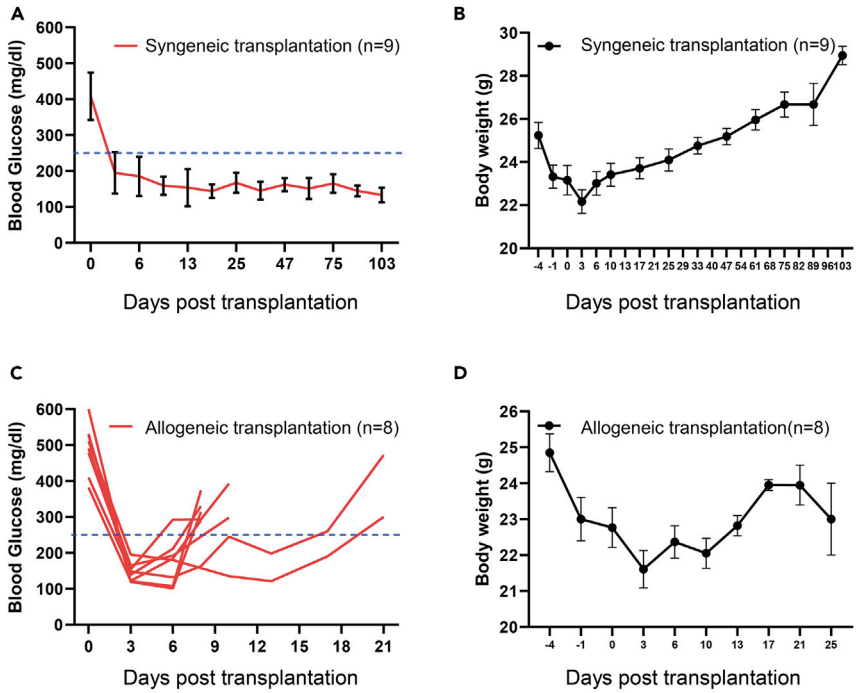

Figure 6. Engraftment of intraportally transplanted islets in syngeneic and allogeneic settings

(A) Survival and function of transplanted islets into streptozotocin diabetic syngeneic recipients (n=9). Blood glucose levels were monitored and graphed at various time points post-transplantation.

(B) Streptozotocin treatment causes weight loss, but the recipient's weight returns to normal and increases after islet transplantation.

(C) Blood glucose levels at various times post-transplantation in allogeneic recipients (n=8).

(D) Body weight loss in allogeneic recipients follows a bimodal curve, an initial decrease due to streptozotocin treatment followed by recovery after islet transplantation and then a decline precipitated by graft rejection and hyperglycemia.

inadequate oxygenation during the early post-islet transplantation period may contribute to islet loss and dysfunction (Suszynski et al., 2014).

TROUBLESHOOTING

Problem 1

STZ caused mortality or lack of consistent diabetes Induction (induction of diabetes by streptozotocin).

Potential solution

The sensitivity to STZ depends on strain of mice, age, injection route, and gender, therefore the dose of STZ should be titrated. In our experience, the injection of 200 mg/kg of STZ by tail vein resulted in non-fasting glucose >350 mg/dL in almost 100% of mice within 3–4 days post injection, but this dose is lethal in NSG mice. Some other critical steps are as follows: protect STZ powder and solution from light by covering the tube containing the STZ sample with aluminum foil. To avoid degradation, STZ should be freshly prepared immediately prior to injection and used up within 15 min.

Problem 2

Uneven pancreatic perfusion or duodenum distension (step 6h).

Potential solution

If the ampulla is too tightly clamped, the branches of the pancreatic duct into the head region are blocked, causing only the tail of the pancreas to be distended and the contralateral region of the

pancreas to be poorly perfused. On the contrary, if the ampulla is not sufficiently clamped, the perfusate enters the duodenum and the pancreas is not adequately perfused. The above-mentioned events can be avoided by precisely localizing the ampulla and clamping it. Air bubbles in the solution or rapid perfusion can also rupture the common bile duct or pancreatic duct, resulting in collagenase outflow and poor pancreas distension, which can impair digestion and reduce islet yield.

Problem 3

Islets appear broken or too much exocrine tissue contamination during handpicking (step 9b).

Potential solution

Over digestion with the enzyme results in islet damage, manifested as broken and loss of intact outer layer. Damage to the islets can be reduced by shortening the digestion time by gently shaking. If excessive exocrine glands adhere to the surface of the islets, this indicates under digestion. This may be due to inadequate perfusion of the pancreas or insufficient digestion time. Therefore, perfusion of the pancreas is critical for optimal digestion. Poor perfusion inevitably leads to low islet yield. it is recommended between 10–17 min with resuspension of digested tissue every minute using a barotropic pipette and microscopic observation to determine the optimal digestion time.

Problem 4

Low islet purity (step 8e).

Potential solution

Islets at the 11%–20.5% interfacial layer contain considerable exocrine cells, other ductal tissues, and cellular debris, making manual handpicking challenging. Islets contaminated with exocrine tissues also release harmful elements during culture, thus affecting islet viability and function. Increasing washing steps can improve islet purity due to preferential sedimentation of islets as compared with non-islet cells. Reducing the mass of tissue loaded on Ficoll gradient can also improve purity of islets.

Problem 5

Hemorrhage in mice after intrahepatic islet transplantation (step 16).

Potential solution

Observe the vessel to be punctured under the microscope (×10), moistened the surface of vessel with saline, and compress the puncture point with a coagulation sponge before pulling out the needle. Increase the compression time from 3 to 5 min and put the coecum back into the abdominal cavity with gentle movements without tugging the tether of punctured vessel. Surgical incision can be enlarged appropriately to facilitate the repositioning of the intestine into the abdominal cavity.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Esma S. Yolcu (esma.yolcu@health.missouri.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate unique code.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2022.101416.

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AUTHOR CONTRIBUTIONS

Methodology, L.Z.; investigation, L.Z. and A.T.; writing – original draft, L.Z.; writing – review & editing, L.Z., H.S., and E.S.Y.; funding acquisition, H.S. and E.S.Y.; supervision, H.S. and E.S.Y.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Bennet, W., Groth, C.G., Larsson, R., Nilsson, B., and Korsgren, O. (2000). Isolated human islets trigger an instant blood mediated inflammatory reaction: implications for intraportal islet transplantation as a treatment for patients with type 1 diabetes. Ups J. Med. Sci. *105*, 125–133. https://doi.org/10.1517/03009734000000059.

Cantarelli, E., Citro, A., Marzorati, S., Melzi, R., Scavini, M., and Piemonti, L. (2013). Murine animal models for preclinical islet transplantation: no model fits all (research purposes). Islets *5*, 79–86. https://doi.org/10.4161/isl.24698.

Delaune, V., Berney, T., Lacotte, S., and Toso, C. (2017). Intraportal islet transplantation: the impact of the liver microenvironment. Transpl. Int. *30*, 227–238. https://doi.org/10.1111/tri.12919.

Kawahara, T., Kin, T., Kashkoush, S., Gala-Lopez, B., Bigam, D.L., Kneteman, N.M., Koh, A., Senior, P.A., and Shapiro, A.M.J. (2011). Portal vein thrombosis is a potentially preventable complication in clinical islet transplantation. Am. J. Transpl. *11*, 2700–2707. https://doi.org/10.1111/j.1600-6143.2011.03717.x.

Khatri, R., Hussmann, B., Rawat, D., Gurol, A.O., and Linn, T. (2018). Intraportal transplantation of pancreatic islets in mouse model. J. Vis. Exp. 57559. https://doi.org/10.3791/57559.

McCall, M.D., Maciver, A.H., Pawlick, R.L., Edgar, R., and Shapiro, A.J. (2011). Histopaque provides optimal mouse islet purification kinetics: comparison study with Ficoll, iodixanol and dextran. Islets *3*, 144–149. https://doi.org/10.4161/isl.3.4.15729.

Merani, S., Toso, C., Emamaullee, J., and Shapiro, A.M.J. (2008). Optimal implantation site for pancreatic islet transplantation. Br. J. Surg. *95*, 1449–1461. https://doi.org/10.1002/bjs.6391.

Shrestha, P., Batra, L., Tariq Malik, M., Tan, M., Yolcu, E.S., and Shirwan, H. (2020). Immune checkpoint CD47 molecule engineered islets mitigate instant blood-mediated inflammatory reaction and show improved engraftment following intraportal transplantation. Am. J. Transpl. *20*, 2703–2714. https://doi.org/10.1111/ajt.15958.

Suszynski, T.M., Avgoustiniatos, E.S., and Papas, K.K. (2014). Intraportal islet oxygenation. J. Diabetes Sci. Technol. *8*, 575–580. https://doi.org/10.1177/1932296814525827.