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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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Protocol
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 Ca²⁺ and Mg²⁺-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 | Sigma-Aldrich | Cat#S0130 |
| 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#25030081 |
| Penicillin-Streptomycin (10,000 U/mL- 10,000 µg/mL) | Gibco | Cat#15140122 |
| 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

- **CS7BL/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#12000122
- **1.5-inch bulldog clamp**
  - Fine Science Tools
  - Cat#1805135
- **Ultra-Fine Point Tweezers**
  - Fisherbrand
  - Cat#AF103
- **30G 1/200 needle**
  - EXELINT International
  - Cat#26437
- **3 mL syringe**
  - BD
  - Cat#309657
- **50 mL tubes**
  - Fisherbrand
  - Cat#DS-539-13
- **100 mm petri dish**
  - Fisherbrand
  - Cat#FB0875712
- **60 mm petri dish**
  - Fisherbrand
  - Cat#FB0875713A
- **Falcon 17 x 100 mm, 14 mL round polypropylene bottom test tubes**
  - Life Sciences
  - Cat#352059
- **7X–45X Stereo Zoom Inspection Industrial Microscope**
  - Amscope
  - Cat#SM-18N
- **Inverted cell culture microscope**
  - Olympus
  - Cat#CX51
- **Nylon Mesh Filters, 500 µm,**
  - Tisch Scientific
  - Cat#ME17264
- **General-purpose water bath**
  - Fisher Scientific
  - Cat#FSGPD02
- **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*258LS
- **Gel-Loading Tips, 1–200 µL**
  - Fisherbrand
  - Cat#20-707-81
- **1 mL syringe**
  - BD
  - Cat#309623
- **Absorbable Gelatin Sponge**
  - Ethicon
  - Cat#1972
- **7X–90X Binocular Stereo Boom Microscope + Ring Light**
  - Amscope
  - Cat#SM-48Y-FRL
- **EVENCARE G2 Blood Glucose Meter**
  - Medline
  - Cat#MPH1540NV
- **EVENCARE G2 glucose strips**
  - Medline
  - Cat#MPH1550

### MATERIALS AND EQUIPMENT

#### Islet wash solution

| Reagent | Final concentration | Amount |
|---------|---------------------|--------|
| FBS | 1% | 5 mL |
| HBSS with Ca²⁺ and Mg²⁺ | N/A | 485 mL |

(Continued on next page)
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.

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**Complete islet culture medium**

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

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

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**iDTZ stock solution (8 mg/mL)**

| iDTZ solution                        | 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.

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**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 CO₂ 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.
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.
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% CO₂.

⚠️ 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 range (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).
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

Ω 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.
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.

\[\text{CRITICAL: To avoid post-transplant bleeding, do not pull too hard on the injected vessel.}\]

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
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),...
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

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.
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.

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