A protocol for islet isolation from mouse pancreas

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Mouse islets are commonly used in diabetes-related studies. Adequate amounts of good quality islets are prerequisites for a reliable investigation. We describe a protocol for islet isolation from mouse pancreas. Three major manipulations are employed in the islet isolation procedure: *in situ* pancreas perfusion with collagenase, pancreas digestion and islet purification. The whole procedure takes 30–45 min for each individual mouse. By using this protocol, a reasonable number of islets can be obtained in a relatively short period of time. This protocol has been proven to be practicable and reproducible. It can be easily followed by individuals who do not have previous experience in the related research field.

INTRODUCTION

The recent era of islet transplantation research began more than four decades ago. In 1967, Lacy's group described a new collagenase-based method to isolate islets, paving the way for future islet experiments both *in vitro* and *in vivo*[1], and eventually leading to a successful islet transplantation to treat patients with type 1 diabetes; since then it has been referred to as the Edmonton protocol[2]. Islet isolation has been a fundamental technique in the field of diabetic research for almost half a century. Achieving good islet isolation is one of the most important factors for reliable islet transplantation studies.

Overview of procedure

Mouse islet isolation from pancreas is a commonly used technique for diabetic investigations. A classical procedure includes three steps: collagenase perfusion, pancreas digestion and islet purification.

Collagenase perfusion is the key step leading to a desired outcome of islet isolation in both quantity and quality. Owing to its special feature of digestive enzyme production, the pancreas is extremely time-sensitive towards islet isolation. Thus, minimizing the variation during collagenase perfusion is expected to improve the process of islet preparation. The common bile duct is the site through which collagenase solution is perfused. Direct puncture[3] and catheterization through gall bladder[4] have been used to insert a needle into the common bile duct. Because of the size and length of the duct certain experience is required to perform such a puncture, and sometimes it is stressful especially to the new technologists. Direct puncture of common bile duct does not allow multiple attempts because of limited length of the duct, and catheterization through the gall bladder works well only when the cystic duct is relatively large. In the present protocol we introduce the joint site of hepatic duct and cystic duct as the puncture route, which is more feasible and controllable especially for the new researchers.

Islet purification is another critical step towards desired preparation. Three different methods can be used to purify islet from collagenase-digested panceatic tissue, Ficoll[2,4,5] and iodixanol[6] gradient separation, filtration[6,7] and magnetic retraction[8,9]. As the magnetic retraction is fit for islet isolation from human and large animals, most investigators make use of two techniques to acquire islets from mouse pancreas. The first technique is the filtration procedure developed by Salvalaggio *et al.*[3], the other technique is the Ficoll method introduced by Bluestone's group[4]. Investigators apply one of these two techniques along with their own individual modifications. Using the filtration procedure described by Salvalaggio *et al.*, mouse islet preparation was generated through the following procedures: clamping the common bile duct, distending the pancreas with collagenase V, mincing the pancreas with surgical scissors, digesting and hand-shaking, and finally filtering through 100 μm nylon cell strainer. An overall purity of ~86% (before hand-shaking) was achieved through this procedure. The Ficoll method has the disadvantage of potential toxic effect on the isolated islets. Our protocol uses a filtration procedure to purify islets from collagenase-digested mouse pancreas. To make the protocol more controllable, our protocol gives a clear description on the critical steps, such as the method of clamping the common bile duct, the location of puncture for collagenase perfusion and the extent of hand-shaking of digested tissue. In order to enhance the islet yield, a smaller pore size (70 μm) cell strainer is used on the premise that reasonable purity is achievable. Over 95% overall purity is readily obtainable in a relatively short period of time by using this protocol.

Results obtained previously using the protocol

This protocol has been used in our recent diabetic research projects[10,11]. Several mouse strains were involved in our studies, such as C57BL/6J, BALB/cJ, NOD, SCID and NOD–SCID. The islet yield ranged from 150 to 450 (islets/mouse) depending on the mouse age and strain. This protocol is straightforward and reproducible. It can be easily followed by individuals who do not have previous experience in this field.

MATERIALS

REAGENTS

- Hank's balanced salt solution (HBSS, GIBCO, cat. no. 14185-052)
- Collagenase XI (Sigma, cat. no. C7657)
- RPMI 1640 (GIBCO, cat. no. 11875)
- L-glutamine (GIBCO, cat. no. 25030081)
- Penicillin–streptomycin (GIBCO, cat. no. 15140122)
- $CaCl_2$ (Sigma, cat. no. C5080)
- FBS (GIBCO, cat. no. 26140087)

- Nembutal (50 mg ml^{-1} solution) ! CAUTION Toxic if swallowed.
- Dithizone (Sigma, cat. no. D5130) ! CAUTION Dithizone is an irritant. When used, avoid inhalation and skin contact.
- Mice (C57BL/6J, 8–12 weeks old) ! CAUTION Experiments involving rodents must conform to National and Institutional regulations.

EQUIPMENT

- Biological hood
- Dissecting microscope
- 37 °C water bath
- Centrifuge (Eppendorf, 5810R)
- 5-ml syringe with 30G1/2-G needle
- Curved hemostatic forceps (125 mm)
- Dissecting scissors (100 mm)
- 70 μm cell strainer (BD Falcon, cat. no. 352350)
- 100 mm Petri-dishes (Corning, cat. no. 430591)
- 50-ml tubes
- 25-ml pipettes
- Wide-open pipette tips
- 200 μl pipette

REAGENT SETUP

Solution I Add $CaCl_2$ (1 mM) into 1× HBSS. ▲ CRITICAL Keep it on ice for no more than 24 h.

Solution II Dissolve collagenase XI (1,000 U ml^{-1}) in 1× HBSS and transfer 5 ml into 50 ml tube for each mouse. ▲ CRITICAL Keep the tubes on ice for no more than 4 h.

Solution III Add L-glutamine (20 mM), penicillin (100 U ml^{-1}), streptomycin (100 μg ml^{-1}) and FBS (10%) into RPMI 1640 medium. ▲ CRITICAL Keep it on ice for no more than 24 h.

PROCEDURE

Surgical operation

1| Inject the mouse with Nembutal (i.p., ~0.05 ml per mouse). Once the animal is fully anesthetized, carry out cervical dislocation and move the mouse into the biological hood.

2| Lay the mouse with the abdominal side facing up and sterilize the skin with 70% ethanol.

3| Make an incision around the upper abdomen to expose the liver and intestines.

Pancreas perfusion and removal

4| As illustrated in **Figure 1**, find the location of the ampulla and clamp it with surgical clamps on the duodenum wall to block the bile pathway to the duodenum.
? TROUBLESHOOTING

5| Take 3 ml of Solution II with a 5 ml syringe mounted with a 30G1/2-G needle. As illustrated in **Figure 1**, insert the needle into the common bile duct through the joint site of the hepatic duct and the cystic duct and reach the middle of common bile duct under the microscope. Distend the pancreas by slowly injecting 3 ml of Solution II.
? TROUBLESHOOTING

6| Remove the pancreas and place it in a 50 ml tube containing 2 ml of Solution II.
? TROUBLESHOOTING

Pancreas digestion

7| Place the tube in a water bath at 37.5 °C for 15 min. Briefly shake the tube two to three times by hand during the incubation.

8| After incubation, shake the tube by hand to disrupt the pancreas until the suspension turns homogeneous.
▲ CRITICAL STEP Once the tissue suspension dissolves to very fine particles, terminate the digestion by going to next step.
? TROUBLESHOOTING

9| Terminate the digestion by putting the tube on ice and adding 25 ml of Solution I. Centrifuge at 290*g* for 30 s at 4 °C and discard the supernatant. Then, resuspend the pellet with 20 ml ice-cold Solution I, centrifuge again at 290*g* for 30 s at 4 °C and discard the supernatant.

Islet purification

10| Resuspend the resulting pellet with 15 ml of Solution I. Pour the resuspended solution onto a pre-wetted 70 μm cell strainer. Wash the tube with 20 ml of Solution I and pour again onto the strainer.

11| Pour another 25 ml of Solution I through the filter.

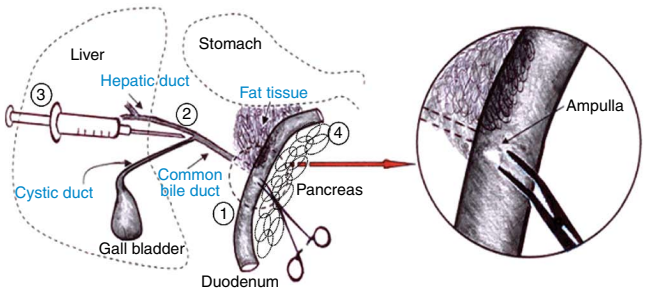

Figure 1 | Procedure of mouse pancreas perfusion and removal. (1) Find and clamp the ampulla with curved hemostatic forceps. As shown in the insert of the figure, the ampulla is located in the duodenum wall near the edge of fat tissues. This step can be done without the aid of the microscope. (2) Insert the needle into the common bile duct through the joint site of the hepatic duct and the cystic duct. (3) Slowly inject the first half of the solution, and then increase the injection speed. (4) Harvest the distended pancreas starting from the duodenum and avoid the cluster of fat tissue.

12| Turn the strainer upside down over a new petri dish and rinse the captured islets into the dish with 15 ml of Solution III.

Islet picking and counting

13| Hand-pick the isolated islets using a pipette with a wide-open tip, count and place the islets in 5% CO_2 incubator at 37 °C.

● TIMING

Steps 1–3, Surgical operation: 3–5 min
Steps 4–6, Pancreas perfusion and removal: 4–6 min
Steps 7–9, Pancreas digestion: 18–20 min
Steps 10–12, Islet purification: 3–5 min
Step 13, Islet picking and counting: 3–7 min

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 1**.

TABLE 1 | Troubleshooting table.

Step	Problem	Possible cause and solution
4	Duodenum distended during enzyme injection	Injected buffer flows to duodenum. As illustrated in the insert of **Figure 1**, the location of the ampulla is characterized by a triangle-shaped milky area on the surface of the duodenum. Make sure to realize the location of the ampulla and reclamp it
5	The common bile duct distended during perfusion	The needle tip is not in the bile duct cavity. To confirm this, the duct does not move with the needle when shifting the needle side to side. Reinsert the needle under the microscope. Fast perfusion can also induce the problem
6	Fat tissue may affect digestion and reduce yield	During pancreas removal, avoid collecting fat tissue. Fat tissue is distinguishable from distended pancreas by color and tissue density
8	Incomplete digestion	As showed in **Figure 2b,e**, some islets are attached to the cluster of exocrine tissue. During hand-shaking, make sure the evenly fine particles being achieved

ANTICIPATED RESULTS

Through using this modified protocol, one can obtain a reasonable number of islets with very good purity from commonly used mice. In most cases, isolated islets are almost 100% free from exocrine tissue before hand-picking

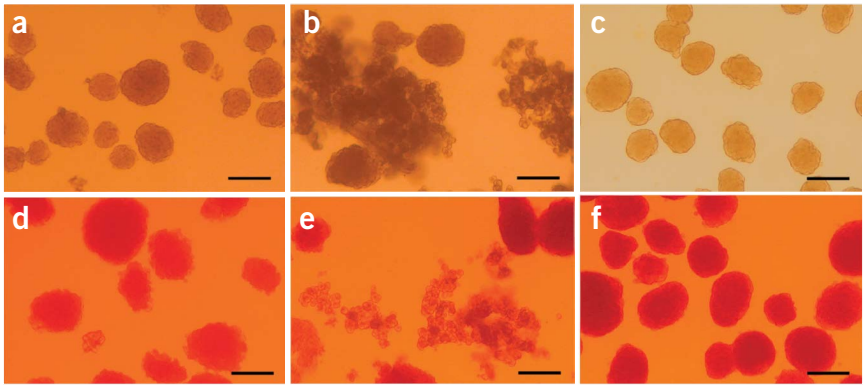

Figure 2 | Isolated mouse islets. The photos show the islets kept in cell culture medium (**a**,**b**,**c**) and the islets after dithizone staining (**d**,**e**,**f**). (**a**,**d**) Islets isolated through the present procedure before hand-picking. (**b**,**e**) Incompletely digested islets for troubleshooting. (**c**,**f**) Purified islets after hand-picking. The photos show the islets kept in the culture medium (top row) and the islets after dithizone staining (bottom row). Scale bars represent 150 μm.

(**Fig. 2**, see panels **a** and **d**). The procedure of hand-picking is carried out mainly for the purpose of quantification. The number of isolated islets varies with animal age and strains. 230–330 islets per mouse were obtained from 8–12 week old C57BL/6J in this protocol preparation. The whole procedure takes 30–45 min for each individual mouse, but the average time spent on each animal can be remarkably reduced when a planed experiment is carried out with a batch of animals. This protocol has been successfully used in our recent studies and has proven to be practicable and reproducible.

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AUTHOR CONTRIBUTIONS All authors contributed equally to this work. Y.-H.Y. and Q.-L.L. conducted reagent and equipment preparation, animal handling and operation. D.-S.L. and L.-J.D. performed the pancreas digestion and islet purification. L.-J.D. wrote the main paper. All authors discussed the results and implications and commented on the manuscript at all stages.

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