Video Article

A Simple Method for Rat Pancreatic Islets of Langerhans Isolation

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URL: http://www.jove.com/video/4192
DOI: doi:10.3791/4192

Keywords: Rat, Pancreas, The islets of Langerhans, Isolation

Date Published: 6/15/2015

Citation: Sojoodi, M., Moslem, M., Baharvand, H. A Simple Method for Rat Pancreatic Islets of Langerhans Isolation. *J. Vis. Exp.* (), e4192, doi:10.3791/4192 (2015).

Abstract

Insulin-dependent diabetes mellitus (type 1 diabetes) is an autoimmune disease resulted from selective destruction of insulin producing cells, beta cells, located in the pancreatic islets of Langerhans. Consequentially, it is a chronic disease characterized by elevated blood glucose levels (hyperglycemia). Chronic hyperglycemia can eventually lead to other complications such as blindness, kidney failure, and amputations. According to the World Health Organization, at least 364 million people worldwide suffer from diabetes. Type 1 comprises 10% of people with diabetes around the world causing significant economic impact on individuals, families and health care systems. Treatments aim to prevent complications of diabetes by controlling blood glucose levels either by daily injection of insulin or by replacement of destroyed beta cells. However, insulin therapy is a far from optimal condition and cannot prevent long-term complications. Since 1967, when Lacy and Kostianovsky introduced a new collagenase based procedure for islet isolation, the achievement of a high yield method has become one of the most favored issues by scientists. There are several factors influencing the islet isolation, *in vitro* culture and *in vivo* transplantation which have to be investigated. With all respect to previous methods and protocols, here we introduce a simple method for isolation of rat pancreatic Islets of Langerhans, including three major steps: (1) collagenase perfusion into the pancreas, (2) pancreas digestion, and (3) the purification of islets using ficoll gradients. This protocol provides an efficient and reproducible method to isolate intact islets with the least amount of contamination to exocrine tissues. Additionally, in comparison with previous methods, our protocol is simple, less intensive, and low-cost. The entire procedure takes 30-45 min which is important for conserving islets viability. Additionally, this procedure can simply be applied by any individual attempting to isolate rat islets of Langerhans for the first time.

Video Link

The video component of this article can be found at http://www.jove.com/video/4192/

Introduction

Pancreatic islet transplantation is a cell-based method in the treatment of diabetes type I patients. This procedure is under numerous clinical trials in different countries, which have promising results for diabetic patients. Meanwhile there are still some challenges in the islet transplantation method including allo-immune reactions, islet engraftment and vascularization, which require broader experimental research in this field to be solved.

The first important step in the research studies in the field of islet transplantation, is pancreatic islet isolation. A reliable investigation depends on adequate and intact islets. Also, a successful islet isolation procedure results in viable purified islets that respond in a manner consistent with their function *in vivo*.

Each islet isolation procedure typically has 3 major steps: (1) collagenase perfusion into the pancreas via the common bile duct; (2) pancreas digestion, and (3) purification of islets using density gradients. Here, we describe a simple, low cost method for isolation of pancreatic islets from rat or mouse, which can be used by any individual requiring a fast simple method for islet isolation.

Protocol

All research and animal care procedures were undertaken in strict accordance with the approval from of the Institutional Review Board and Institutional Ethical Committee.

1. Surgical Procedure

1. Anesthetize animal by IP injection of anesthetizing solution (ketamine/xylazine).
2. Ensure that the animal is properly anesthetized by toe pinch.
3. Lay the animal in appropriate position so the surgeon can visualize the abdomen. It is recommended to use vet ointment on eyes to prevent dryness while under anesthesia

4. Spray 70% ethanol on the animal to reduce the chance of contamination during surgery. Moreover, surgical areas should be completely shaved to avoid contamination of the wound through further contacts. It is recommended to immobilize the animal.
5. Open the abdomen in a V shape, starting from the lower abdomen in two directions and extend the scalpel to the lateral portions of the diaphragm in order to expose all organs in the peritoneal cavity.
6. Displace the internal organs, such as intestine and stomach, to the right side.
7. Secure the liver with gauze or swab.
8. Find the common bile duct (CBD), which is extended from the liver to the intestine.
9. Locate where the CBD enters the small intestine.
10. Using a Bulldog clamp, clamp the CBD near its junction with the small intestine. Be careful not to occlude the pancreatic duct. Clamping the duodenum on either side of the junction with the CBD is another alternative.
11. Fill a 5 ml syringe with solution I (enzyme solution).
12. By a micro Scissor make a scission in the CBD at the most superior portion of the CBD.
13. Inject 4-5 ml of solution I into the CBD for full inflation of the pancreas. Full inflation of the pancreas in this step greatly influences the final yield. Remove the pancreas and place it into a 50 ml conical tube. To remove the pancreas, begin at the point where the CBD is attached to the duodenum. Then, cut the CBD from its attachment to liver. Continue removing the pancreas from the stomach until the spleen is reached. It is better not to remove the pancreas from spleen at first since the spleen can be used as a handle. Remove the spleen when the tissue (pancreas) is to be placed into the conical tube that contains 5 ml of solution I (Collagenase solution).
14. Keep the conical tube that contains tissues on ice until the digestion procedure.
15. After removing the pancreas make sure that animal is still anesthetized. In this case you can use standard euthanasia methods for rodents such as Carbon dioxide (CO_2) inhalation, cervical dislocation or decapitation to euthanize the animal. Please keep in mind that euthanasia must be done under anesthesia.

2. Digestion and Purification Procedure

1. Incubate tissues for 20 min at 37 °C in a water bath. Incubation time varies among different lots of enzymes, but is usually 15-20 min.
2. Every 5 min remove the tube, shake firmly 2-4 times, and then return the tube to the water bath.
3. After the incubation time is completed, fill the tube with 10 ml of solution II to dilute Collagenase and stop the digestive process.
4. Shake the tube vigorously for 30 sec to mechanically digest the rest of the exocrine tissues and to complete tissue separation.
5. After mechanical digestion, the mixture should be homogenous and resemble to the pea soup. Fat tissue is also isolated with the pancreas and will remain undigested; therefore it is normal to see undigested pieces of fat in the final soup.
6. Pour the soup through the sieve. In this step, undigested tissues cannot pass through the strainer therefor digested tissue can be collected.
7. Wash the surface of strainer with solution II to wash all the remaining islets.
8. Immediately transfer the filtered solution into a 50 conical tube.
9. Centrifuge for 1 min at 1000 rpm.
10. Discard the supernatant.
11. Resuspend the pellet in 10 ml solution II.
12. Centrifuge for 1 min at 1000 rpm.
13. Repeat steps 11-12 for two more times.
14. Resuspend the pellet in 20 ml ficoll (lymphodex) solution. Resuspend the tissue completely by proper pipetting and make sure that there are the least tissue clamps after pipetting. Tissue should become almost homogenous with the ficoll. If not, attached exocrine tissues to islets affect the islets' density and make them to stand in wrong gradient layer.
15. Fill the 20 ml syringe with solution III.
16. Turn the tube to a 45 degree angle and add solution III slowly via a syringe. Avoid mixing the medium and ficoll with each other. As a result two separate phases will form, the upper one is ficoll and the lower one is medium
17. Centrifuge at 2000 rpm for 10 min with no acceleration and breaking speed.
18. After the centrifuge is completed, the isolated islets stand in the intermediate phase between the ficoll and the medium. Due to toxicity of ficoll for cells this step should performed quickly to avoid cell apoptosis. Transfer the collected islets to another 50 ml conical tube.
19. Resuspend the collected islets in 10 ml of solution III. It is helpful to separate the collected fine exocrine tissue, if any, also this procedure helps diluting the pulled up ficoll.
20. Centrifuge for 1 min at 1000 rpm.
21. Decant the supernatant and replace it with 10 ml of solution III.
22. Repeat steps 19-21 twice more.
23. Transfer the islets to a sterile petri dish under a culture hood to incubate or pick up the islets under a dissecting or light microscope. Use a suspension culture dish so the islets do not stick as they would in treated tissue cultures.

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

Representative Results

A few other cell types, such as acinar and ductal tissues, observed around the islets, suggests the efficient isolation of islets. The islet purity ranged from 80 to 95% after the final hand picking and transferring into the culture dishes (**Figure 1**). Moreover, the protocol presented here offers an opportunity to acquire reasonable number of islets, in addition to less time for each rat, 30-45 min.

To evaluate morphology of isolated Islets of Langerhans and expression of markers, we analyzed the islets for Insulin and Glucagon protein expression (**Figure 2**). This staining represents unique cytoarchitecture of rodent's pancreatic islets, the vastly predominating β cells which are surrounded by a mantle of α cells.

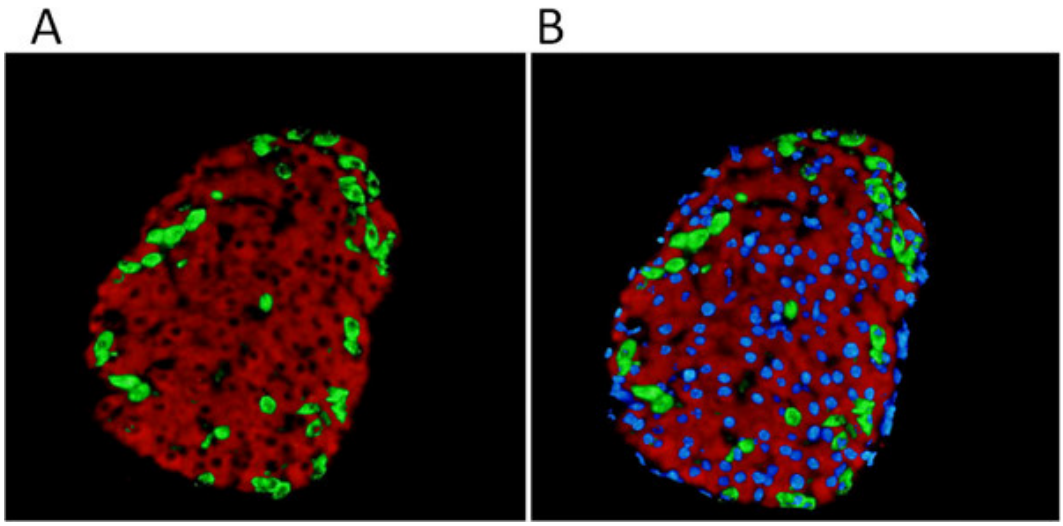

Figure 1. The isolated islets of Langerhans. The morphology of freshly isolated islets (A and B)

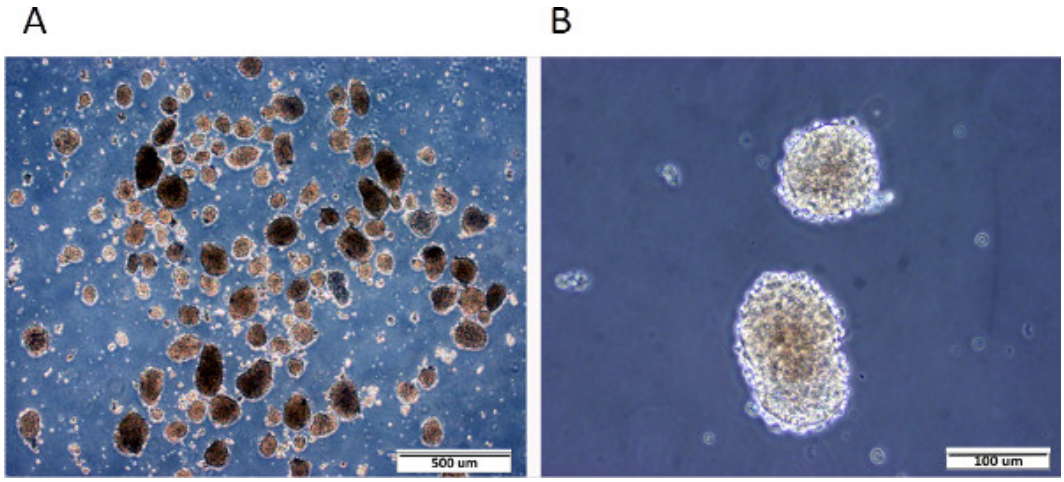

Figure 2. The characterization of isolated islets. (A) Immunofluorescence staining for expression of Insulin (red) and Glucagon (green). (B) Nuclei were stained with DAPI (blue). All magnifications: ×20

Step A: Surgical procedure	Problem	Possible cause	Solution
10	Finding the place where CBD enter the small intestine is difficult.	Junction cannot be visualized.	• Clamping the duodenum on either side of the junction with the CBD is also an option. • Postpone step 10 after injecting collagenase for the first time. At this time, entering the solution into the intestine is completely obvious. Clamping the duodenum at the place where collagenase enters the duodenum.
12	The CBD is cut during making scission.	Using inappropriate scissors.	Make another session at the lower part. If it is destroyed, an alternative method involves directly injecting collagenase into the numerous lobes of the pancreas.
13	The CBD is distended during injection.	• The needle tip is not in the bile duct cavity. • Fast and strong perfusion.	• Reinsert the needle under the microscope. • Slow the perfusion rate. Be patient!
14	Intestine and blood vessels rupture, blood and excrement contamination.	Vessel and intestine rupture during pancreas removal.	Wash the isolated tissues with solution II before transferring to conical tube which contains 5 ml collagenase.

Table 1. Troubleshooting Table

Collagenase solution (solution I)	Dissolve 20 mg Collagenase V in 10 ml HBSS 1x to a final concentration of 2 mg/ml
	Sterilize by filtration through a 0.2 µm filter
	Maintain on ice until further processing
Washing solution (solution II)	Combine the following chemicals,45 ml HBSS 1x , 5 ml FBS and 500 µl of penicillin/Streptomycin
	Sterilize by filtration through a 0.2 µm syringe filter
	Maintain on ice until further processing
Culture media (solution III)	Combine the following chemicals,45 RPMI , 5 ml FBS and 500 µl of penicillin/Streptomycin
	Sterilize by filtration through a 0.2 µm syringe filter
	Maintain on ice until further processing
Anesthetic solution	Mix 2 ml of ketamine and 1 ml of xylazine
	The dosage of ketamine/xylazine used to induce anesthesia is typically 0.005 ml/g body weight.

Table 2. Reagent setup

Discussion

In vitro culture of pancreatic islet of Langerhans is a useful tool by which investigators can monitor and analyze biological and also pathophysiological process involved in endocrine pancreas. The isolation procedure is complicated because of enzymatic approach for digestion of fresh pancreas and needs to be optimized. Gradual delivery of collangenase to pancreatic tissue via common bile duct (CBD) is crucial to have more islet survival and more efficient isolation. More previously, isolation procedures were mostly based on enzymatic digestion of pancreatic pieces in collagenase solution. However perfusing pancreas through common bile duct will lead to a well-organized access to islets all over the pancreas. Moreover by perfusion digestion, the interaction between collagenase and connective tissue surrounding the islets will be more intimate and more efficient to overcome mechanical damages and achieve higher isolated islet yield rather than cutting the pancreas into pieces.

There are two significant check points in isolation procedure. First the collagensase activity, purity, and formulation that firmly influence the islet count and survival. Here we used Collagenase type V, however for an appropriate isolation the composition of collagenase and other enzymes in

each isolation must be controlled for the task with specific purpos. Moreover, since endotoxin activity can affect islet viability, be sure to have no endotoxin presence/activity contaminating the collagenase.

Another significant point is using a density gradient to purify islets from exocrine acinar tissue. As the exocrine pancreas secretes various digestive enzymes, endocrine islet isolation from pancreatic acinar tissue is highly demanded. Although using different commercially available ficoll helps purifying islets immediately, the final purity depends on the animal strain and the characteristics of density gradients. So further purification of islets from acinar tissue is often needed to increase islet purity prior to culture. Our protocol includes using a dissecting microscope to identify the islets, then manually picking those islets from one suspension culture dish into a second dish containing culture medium. Sometimes more purification steps are needed. Take care about contamination and pH changes of medium during hand picking procedure. Transfer the Petri dish, containing the islets into the incubator immediately.

Our protocol is successful method for isolating rat pancreatic islets of Langerhans; however, it can be used in mice as well. The method of collagenase and ficoll administration, as well as digestion time should be optimized by researcher with careful experimentation to the capabilities of the laboratory and the specific aim of the study. In addition, we provided troubleshooting table (**Table 1**), which can help novice researchers to optimize the procedure.

Disclosures

We have nothing to disclose.

Acknowledgements

The authors gratefully acknowledge Hamideh Aghajani, Keynoosh Khaloughi, Ghazaleh Hajmousa, Abolfazl Kheimeh for their critical comments and technical supports. This work was funded by a grant from Royan Institute.

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