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Selective Osmotic Shock (SOS)-Based Islet Isolation for Microencapsulation

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Abstract

Islet transplantation (IT) has recently been shown to be a promising alternative to pancreas transplantation for reversing diabetes. IT requires the isolation of the islets from the pancreas, and these islets can be used to fabricate a bio-artificial pancreas. Enzymatic digestion is the current gold standard procedure for islet isolation but has lingering concerns. One such concern is that it has been shown to damage the islets due to nonselective tissue digestion. This chapter provides a detailed description of a nonenzymatic method that we are exploring in our lab as an alternative to current enzymatic digestion procedures for islet isolation from human and nonhuman pancreatic tissues. This method is based on selective destruction and protection of specific cell types and has been shown to leave the extracellular matrix (ECM) of islets intact, which may thus enhance islet viability and functionality. We also show that these SOS-isolated islets can be microencapsulated for transplantation.

Keywords

Islets; Nonenzymatic; Digestion; Microencapsulation; Alginate; Diabetes; Transplantation

1 Introduction

Type 1 Diabetes Mellitus (T1DM) is a condition of absolute deficiency of the beta cell component of the pancreas. Currently, beta cell replacement is considered the gold standard for the treatment of T1DM [1]. The two forms of beta cell replacement procedures currently in clinical use are whole pancreas and islet transplantations. Islet transplantation requires isolation of the islets from the pancreas. Currently, this process involves enzymatic digestion of the pancreas which has significant harmful effects to the islets and the extracellular matrix (ECM) surrounding them. Although the architecture and physical interactions within and surrounding islets are complex and not completely understood, there is significant evidence that islets, like other tissues, are also heavily influenced by cell–ECM interactions [2]. In mature, intact islets, interactions with ECM or synthetic matrix materials have been shown to regulate cell survival, proliferation, and insulin secretion, and aid in the preservation and restoration of spherical islet morphology [2, 3]. Similarly, ECM-based materials have been shown to regulate survival, proliferation, and insulin secretion in purified β -cell cultures, while incompletely isolated islets that retain some of their native ECM have been shown to have markedly reduced apoptosis rates and significantly greater function with respect to insulin response in vitro than completely isolated and purified islets. This nonenzymatic method, first described by Atwater et al., uses rapid osmosis to isolate islet cells from the

rest of the pancreatic cells [4]. This is possible because the beta cells of islets contain glucose transporters (Glut 2). These transporters allow islet cells to quickly adapt to different levels of glucose, process it, and produce insulin in response to the prevailing glucose concentration [5]. When islets are exposed to high glucose concentrations, islet cells take up the glucose via Glut-2 transporters in the membrane in order to equilibrate the osmotic strength of the external solution, whereas acinar cells rapidly lose water in order to equilibrate internal and external solutions, and this causes the cells to shrink. Acinar cells take up potassium ions to recover cell volume and to adapt to the extracellular osmotic strength. When the glucose solution is removed, the islet cells lose the extra glucose through Glut-2 transporters. However, acinar cells rapidly take up water to dilute the hypertonic intracellular solution, and this causes the cells to swell. The acinar cells swell so fast that they burst [4]. Therefore, we are able to isolate the beta cells from the rest of the pancreatic tissue using this nonenzymatic digestion method.

2 Materials

2.1 Equipment

1. Four sterile disposable 600 mL Beakers [Fisher Scientific; Cat. No. 01-291-6].
2. One Sterile 400 mL glass Beaker [Pyrex; Cat. No. 1000].
3. One Sterile basic Surgical Kit [World Precision Instruments; Cat. No. 503407].
4. One sterile Instrument Tray [Polarware; Ref. # E2306].
5. 2×½ in. Full-Size Heavy Weight Steam Table Pan [Polarware; Ref. # S20122].
6. One 500 µM Sterile Stainless Steel U.S. Standard Test Sieve (4 in. diameter, 2 in. depth) [Cole-Parmer].
7. One Sterile Stainless Steel Bowl 6 5/8 in. × 2½ in. [Polarware; Ref. # 12A].
8. One 150 × 25 mm Suspension Culture Dish (Non-Tissue Culture Treated) [Corning; Cat. No. 430597].
9. One 60 mL Syringe [BD; Ref. No. 309653].
10. One 60 mL Piston/Irrigation Syringe with Protective Cap [Covidien Ref. # 68000].
11. One Package of 50 mL Conical Tubes [BD; Ref. # 352070].
12. Scale (Adventure Pro) [Ohaus; Cat. No. AV201c].
13. Densito 30PX Densitometer [Mettler-Toledo; Mfr. No. 51324450].

2.2 Reagents

1. 1 L (2× 500 mL Bottles) Sterile PBS [Hyclone, Cat. No. SH30028.02].
2. 1 M HEPES Buffer Solution [Cellgro; Cat. No. 25-060-C1].
3. Glucose [Sigma; Cat. No. G7021-1KG].

4. RPMI-1640 Medium [Sigma; Cat. No. R1383–1L].
5. 10 % Betadine [Fischer; Cat. No. NC0304148].
6. 76 mL Optiprep™ [Sigma Cat. No. D1556].
7. 390 mL CMRL-1066 Medium [Fischer; Cat. No. 15–110-CV].
8. 100 mL Fetal Bovine Serum.
9. 5 mL Penicillin/Streptomycin.
10. 339 mL Waters IGL Cold Storage Solution [Hospital Pharmacy Man. No. Ccs/1000/US].

3 Methods

3.1 Preparation of Reagents

1. 300 mM RPMI (100–200 mL per pancreas).
 - a. Reconstitute RPMI in 990 mL of ultrapure H₂O.
 - b. Add 10 mL of 1 M HEPES Stock.
 - c. Add 54 g Glucose and allow to dissolve on stir plate.
 - d. Adjust pH to 7.4.
 - e. Filter Sterilize and store at 4 °C.
2. 0 mM RPMI (about 1 L per pancreas).
 - a. Reconstitute RPMI in 990 mL of ultrapure H₂O.
 - b. Add 10 mL of 1 M HEPES Stock.
 - c. Adjust pH to 7.4.
 - d. Filter Sterilize and store at 4 °C.
3. CMRL Culture Media.
 - a. Start with 390 mL of CMRL-1066 Medium.
 - b. Add 5 mL of 1 M HEPES Stock.
 - c. Add 5 mL of Penn/Strep.
 - d. Add 100 mL of FBS.
 - e. Adjust pH to 7.4.
 - f. Filter Sterilize and store at 4 °C.
4. 265 mL Optiprep (1.10 g/mL).
 - a. Calibrate densitometer according to user's manual.
 - b. Add 214 mL of IGL (1.045 g/mL) and 51 mL of Optiprep (1.32 g/mL) to a plastic beaker.

- c. Mix on shaker for 30 min.
 - d. Take a 1.5 mL sample for density measurements.
 - e. Measure density of sample then adjust concentration by adding IGL or Optiprep until it is 1.10 g/mL.
5. 150 mL Optiprep (1.09 g/mL).
 - a. Calibrate densitometer according to user's manual.
 - b. Add 125 mL of IGL (1.045 g/mL) and 25 mL of Optiprep (1.32 g/mL) to a plastic beaker.
 - c. Mix on shaker for 30 min.
 - d. Take a 1.5 mL sample for density measurements.
 - e. Measure density of sample then adjust concentration by adding IGL or Optiprep until it is 1.09 g/mL.
 - f. Filter Sterilize and store at 4 °C.

3.2 Isolation of Islets

1. Harvest pancreas from animal/donor into container of cold PBS.
2. Remove as much viscera as possible from organs on small surgical plate (on ice or placed on ice packs) using blunt dissection with forceps and shears.
3. Weigh tissue in sterile stainless steel bowl.
4. Rinse with 500 mL of ice cold PBS.
5. Incubate in 200 mL of 10 % ice cold betadine for 5 min in 500 mL beaker.
6. Rinse with 500 mL ice cold PBS X3 into 500 mL beakers.
7. In large surgical plates, finely mince tissue (at least $<0.25 \text{ cm}^2$) and place in 50 mL conical tubes (*see* Notes 1 and 2).
8. Expose to 300–600 mM glucose RPMI medium for 20 min on ice.
 - a. Use a 1:1 ratio of tissue to high glucose RPMI.
 - b. Fill up to 50 mL mark on conical tubes.
9. Centrifuge for 2 min at $200 \times g$ 4 °C.
10. Quickly decant supernatant and add 0 mM glucose RPMI.
11. Gently invert tubes to expose cells to low glucose media.
12. Centrifuge for 2 min at $200 \times g$ 4 °C.
13. Repeat **steps 10–12** twice and decant 0 mM RPMI Solution.
14. Remove tissue from 50 mL conical tubes and place in a sterile 500 mL beaker (*see* Note 3).

15. Add 50 mL of 0 mM RPMI.
16. Using an irrigation syringe, gently disrupt tissue by suctioning up and down for approximately 5–10 min (*see* Note 4).
17. If possible maintain the 500 mL beaker on ice or frozen aluminum beads while dissociating.
18. Once the tissue has been dispersed enough to easily pass through the irrigation syringe switch to a 60 mL syringe with a leur lock and continue disrupting tissue for an additional 5–10 min. Maintain temperature at ~4 °C.
19. Rinse tissue through 500 µM strainer into a sterile 500 mL glass beaker.
20. Take the remaining tissue which did not pass through the 500 µM filter and place back in the 500 mL beaker with 50 mL of additional 0 mM RPMI.
21. Repeat dissociation for an additional 5 min.
22. Rinse tissue through 500 µM strainer into 500 mL glass beaker from **step 19**.
23. Repeat **steps 20–22**.
24. Take the filtered tissue located in the glass beaker and pipette into 50 mL conical tubes.
25. Centrifuge for 3 min at 100×*g* (accel/decel = 4, 4 °C) (*see* Note 5).
26. Aspirate supernatant and fill conical tubes with fresh 0 mM RPMI.
27. Repeat **steps 25** and **26** twice, on the last wash, do not fill with fresh RPMI (*see* Note 6).
28. Layer a 50 mL conical tube with 30 mL 1.10 g/mL Optiprep, 8 mL 1.09 g/mL Optiprep, then 10 mL 1.045 g/mL IGL (*see* Note 7).
29. Syringe tissue pellet gently on top of layered Optiprep.
30. Centrifuge for 3 min at 375 g (accel/decel = 1, 4 °C).
31. Purified islets will be located in the middle layer containing 1.09 g/mL Optiprep (*see* Note 8).
32. Carefully use a syringe with a large opening to collect purified islets (*see* Figs. 1 and 2) (*see* Note 9).
33. Resuspend purified tissue pellet in 25 mL of RPMI Culture Media and plate into one large non-tissue culture treated petri dish.
34. Place in 37 °C, 5 % CO₂ incubator and culture overnight.
35. Change media in the morning.

3.3 Microencapsulation of Islets

Encapsulation of islets is performed using an alginate-derived method we developed in our lab [6].

1. The islets are suspended in the alginate solution then loaded into the droplet generator.
2. The droplets form while falling into a CaCl_2 bath for crosslinking for 15 min.
3. The encapsulated islets are collected and washed with normal saline (*see* Fig. 3) (*see* Note 10).
4. Following washing, the microbeads are incubated in PLO for 10–20 min.
5. Repeat **step 38**.
6. The alginate core is liquefied by incubating in 55 mM Sodium Citrate for 2 min.
7. Repeat **step 38**.
8. The microbeads are incubated in alginate solution for 30–45 min followed by Calcium-supplemented saline washes to cross-link the outer layer.
9. Encapsulated islets can then be implanted for in vivo studies.

4 Notes

1. Taking extra time in this step will make mechanical dissociation with syringe much easier and quicker.
2. It is also possible to place minced tissue all in one 500 mL beaker with 300 mM glucose RPMI. If using 50 mL conical tubes, only place about 10–15 mL of tissue per tube.
3. If pancreas is small (rat or mouse), placing in a smaller container such as the stainless steel bowl used for weighing (2.1.7) may allow for quicker dissociation.
4. Slowly push out tissue. High pressures can occur if nozzle is filled with large pieces of tissue and can quickly spray tissue out of beaker if pushed too aggressively. Holding the syringe still and twisting the pump while pushing tissue out can control flow better. This is also true for 60 mL syringe.
5. Acceleration/Deceleration numbers may differ between centrifuge equipment. We used a machine with a scale from 1 to 9 with 1 being the slowest and 9 being the fastest.
6. Tissue pellet should be about 2 mL, if larger, adjust Optiprep volumes to allow for more tissue. Ratios of layers should remain the same.
7. The Optiprep density gradients are very small meaning that they will mix easily if not added slowly. Add Optiprep at a rate of <2 mL/min so the layers are well defined.
8. If a larger yield is required, collect from the top of the 1.045 gradient as well.
9. 25 mL or 50 mL syringes have larger openings which allow for islets to be collected without getting stuck on the tip. If many islets are getting stuck inside

the syringe chamber, collect smaller volumes of islets at a time. Again, the density gradient is very sensitive, move slowly so the layers are not disturbed.

10. Do not centrifuge microbeads, as they are fragile. Allow microbeads to settle via gravity then aspirate.

Disclosure

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Fig. 1.
Rat islet isolated using SOS protocol with 300 mM. Islet is stained *red* with dithizone to measure viability

Fig. 2. Human islet isolated using SOS method with 300 mM glucose. The islet surrounded by pancreatic ECM is stained *red* using Dithizone to assess viability

Fig 3.
Rat islet isolated with SOS method. Islets are purified, encapsulated in alginate, then stained *red* with Dithizone to assess viability