ISLET EMBEDDING FOR HISTOLOGY

I. Definitions

1. **Islet Equivalent (IEQ):** An islet with a diameter of 150 µm determined mathematically by compensating for islet shape.

II. Equipment and Materials

1. Equipment

   - Adjustable tilt rocker (LabNet)
   - Microscope (Olympus SZX12)
   - Digital Camera (Olympus DP80)
   - -80°C Freezer (Thermo Scientific)

2. Supplies and Materials

   - PBS (phosphate buffered saline) with no Ca/Mg, 1X (Invitrogen 14190-144)
   - Collagen I stock (BD 354249)
   - DMEM, 5X (Dulbecco modified Eagle's minimal essential medium, Gibco 31600-034)
   - HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid, Sigma H0887)
   - $NaHCO_3$ (sodium bicarbonate, Sigma S8761)
   - Sucrose (Fisher Scientific BP220-1)
   - 16% Paraformaldehyde (Electron Microscopy Science 15710)
   - 1.5mL Centrifuge Tube (Fisher Scientific 05-408-129)
   - P-200 Pipet Tips, Sterile (Fisher Scientific P-2069)
   - Wide mouth pipet tips for transferring islets (Fisher Scientific 13-811-164)
   - 96-well Plate (Falcon 353072)
   - Needle, 28G, insulin syringe (Exel International 26027)
   - 12-well plate (Fisher Scientific 351143)
   - Cryomolds (Tissue-Tek 4557)
   - OCT (Optimum Cutting Temperature compound, Tissue-Tek 4583)
   - Plus Gold Slides (Fisher Scientific 15-188-48)

III. Procedures

1. Islet Immobilization and Fixation

   - Prepare Collagen I working solution (1mL) by combining 375 µL Collagen I stock, 355 µL sterile water, 20 µL HEPES, 50 µL $NaHCO_3$, 200 µL 5X DMEM.
   - Using a P-1000 pipette transfer an aliquot of islet suspension containing 500 IEQs into a 1.5 mL centrifuge tube and centrifuge at 1000 rpm for 1 min. Aspirate the supernatant. If islet medium is rich in serum, wash the islets once with 1X PBS.

- Add 150 μL of Collagen I working solution to the islet pellet and transfer the mixture into a 96-well plate. Place the plate in a tissue culture incubator set to 37°C. Incubate for 90 min and Collagen I will form into a gel. While gel is solidifying, islets will settle by gravity at the bottom of the gel and form a monolayer.
- Place 96-well plate on ice and add ice-cold 4% paraformaldehyde/1X PBS on top of the gel to fill up the well. Fix for 15 min on ice.
- Using a 28 G needle (0.3 mL insulin syringe) loosen up the gel containing islets from the sides of the well. Then, using a fine spatula transfer the gel into a 12-well plate containing 3 mL of ice-cold 4% paraformaldehyde/1X PBS per well.

  Note: Be very careful while you do this because the gel is relatively soft.

- Fix the islets on ice for an additional 45 min under very mild agitation using an adjustable tilt rocker (low setting).
- Aspirate the fixative (mild setting) and wash the gel with 3 mL of 1X PBS on ice for 20 min under very mild agitation using an adjustable tilt rocker (low setting).
- Repeat 2 times.

2. Islet Embedding and Sectioning

- After the last wash, add 3 mL of 30% sucrose/1X PBS. Allow the gel to equilibrate for 2-3 hours or overnight at 4°C.
- Lift the gel from the dish using a fine spatula and place it into a standard cryomold that is coated with OCT compound.

  Note: While doing this, orient the gel such that the bottom of the gel (that is where the islets are) is facing the bottom of the cryomold.

- Take a photo of the gel on an Olympus SZX12 microscope at 16x. Example of islets embedded in Collagen I is shown in ***Figure 1***. Fill cryomold to the top with OCT.
- Place the embedded gel on dry ice. When OCT compound is frozen, wrap the block in pre-labeled aluminum foil, place it a zip-lock bag, seal and store at -80°C.
- Cut 8-μm cryosections and mount two sections per slide on *Plus Gold* slides. Transfer slides and blocks on dry ice back to the HIPP and store in a -80°C freezer.
- Enter slides and blocks into the Vanderbilt HIPP electronic inventory system.

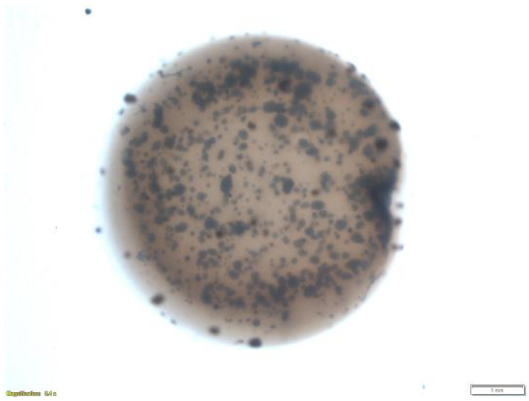

Figure 1. Islets immobilized in Collagen I prepared for embedding and histology.

IV. Data Storage and Reporting

1. Store data in the appropriate server location(s).
2. Annotated images may be uploaded to the HPAP database
3. Document any deviations from this protocol that occurred.

V. References

1. Dai C, Brissova M, Hang Y, Thompson C, Poffenberger G, Shostak A, et al. Islet-enriched gene expression and glucose-induced insulin secretion in human and mouse islets. Diabetologia. 2012 Mar;55(3):707–18. PMCID: PMC3268985
2. Guo S, Dai , Guo M, Taylor B, Harmon JS, Sander M, et al. Inactivation of specific β cell transcription factors in type 2 diabetes. J Clin Invest. 2013 Aug;123(8):3305–16. PMCID: PMC3726150