HUMAN ISLET DISPERSION AND PLATING FOR INTRACELLULAR CALCIUM FLUX AND ELECTROPHYSIOLOGY EXPERIMENTS

I. Reagents/ Buffers/ Materials Needed For Experiment

1. Water-jacketed 5% CO_2 injected 37°C incubator
2. Islet Growth Media
    a. RPMI-1640 (Sigma R1383-10X1L) 8.4g/L
    b. Sodium Bicarbonate 2g/L
    c. 10% FBS
    d. 1% penicillin-streptomycin-amphotericin B solution
    e. 1% Glutamine (2mM Stock)
    f. 10mM Glucose
    g. Adjust pH to 7.0 and sterile filter
3. Glass Bottom Dish 29mm Dish with 10mm Bottom Well (D29-10-1.5N In Vitro Scientific #1.5)
4. 6-well plate (not tissue culture treated)
    a. Poly-lysine placed in 2 wells of 6-well plate
    b. untreated coverslips in 4 wells of for 6-well plate
5. 0.25% Trypsin-EDTA (Invitrogen 25200-056)

II. Procedure

1. Add a minimum of 500 islets to two 15-ml conical tubes
2. Pellet islets for 16 seconds at full speed on bench top centrifuge (International Equipment Company. Model HN)
3. Aspirate and resuspend first islet pellet with 400µl of Islet Growth Media (Whole Islet Tube)
4. Aspirate second islet pellet and resuspend in 250ul 0.25% Trypsin-EDTA (Dispersed Islet Tube)
5. Pipet up and dowun ~30X
6. Continue trypsinizing islets until 70-75% of material is a single cell suspension
7. Inactivate trypsin by adding 9ml of Islet Growth Media
8. Pellet dispersed islets for 45 seconds at full speed on bench top centrifuge (International Equipment Company. Model HN)
9. Aspirate and resuspend Dispersed Islet Tube pellet with 300µl of Islet Growth Media
10. Gently pipet islets in Whole Islet Tube up and down 5-10X to mix and apply 50µl to the center of each glass bottom dish (4 per experiment-Calcium Imaging)
11. Gently pipet islets in Whole Islet Tube up and down 5-10X to mix and apply 50µl drop wise and scattered throughout coverslip (3 per experiment-Electrophysiology)
12. Gently pipet dispersed islets in Dispersed Islet Tube up and down 5-10X to mix and apply 50µl to the center of each glass bottom dish (2 per experiment- Calcium Imaging)

13. Gently pipet dispersed islets in Dispersed Islet Tube up and down 5-10X to mix and apply 50µl drop wise and scattered throughout coverslip (3 per experiment-Electrophysiology)
14. Gently add 2ml of Islet Growth Media to each glass bottom dish
15. Holding the coverslip down with a clean tip or Pasteur pipette, gently add 3ml of Islet Growth Media to each well of the 6-well plate
16. Let islet material recover in 37°C incubator for 48 hours prior to functional assays.