Seeding HUJI diamond devices

Time expected

1. 4 h Device sterilization (can be done before)
2. 1 h Matrigel prep (can be done before)
3. 1.5-2 h Seeding

Device sterilization

Put 0.2mL ethanol in the device well, in the device lid, enclose all in a container and place for 4h-overnight at 80 C to let the ethanol evaporate

Coating

**It is important to coat just the diamond, so place a small enough drop on the top, which does not spill*
1. Put a 15-20 ul drop of matrigel on the top of the square diamond, if its too much, do not push it
2. Put very carfully into incubator to avoid spilling the matrigel 3. Let incubate for 30-45 min at 37C in incubator

Replating cells

1. Prepare Enzyme T in Buffer X from Miltenyi dissociation kit
2. Wash cells with PBS (1mL per 12 well)
3. Add 400 ul of Enzyme T to the well
4. Let it work for 10 min. at 37C
5. Wash down with 2mL of 10% FBS in PBS (or media)
6. Collect by pipetting to dissociate cells
7. Take 10 ul to count
8. Spin at 300 rcf for 3-5 min
9. Aspirate and dissolve to final concnetration of 5 million cells per 1 ml of Replating media
10. Aspirate the Matrigel from the diamond, not spill
11. Take 10 ul of 5e6/ml solution and gently deposit on the diamond, do not spill
12. Let adhere for 30 - 60 min at 37C
13. Gently fill with the rest of the media (0.5 ml) once the cells are attached

Follow up

1. On second day change media to normal RPMI with insulin
2. change media every other day

Media and solutions

Ethanol

Mix 0.3 ml ddH20 and 0.7 ml 96% EtOH

PBS

Should be stock in the lab

Matrigel

Matrigel aliquot thawed on ice for 1 hour
1 aliquot per 5 ml of cold RPMI media (aka MG2x)

Enzyme T

Dilute Enzyme T 1to10 in Buffer X (400 ul per 12 well)

Collection Buffer

Dilute FBS in PBS to 20% (make 5 ml, 1 ml FBS plus 4 ml PBS)

Replating media

Add KOSR and Rock Inhibitor to RPMI 1640 with insulin (0.5 ml KOSR, 2.5 ul RI, 4.5 ml RPMI plus B27)

Images:

MG drop on the diamond