Dedifferentiated Pancreatic islets

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The purpose of islet dedifferentiation is to differentiate pancreatic islet cells to hIPCs (human Islet Precursor Cells), HIPCs are highly proliferative and exhibit a mesenchymal phenotype before transition into epithelial clusters containing cells expressing insulin or glucagon. (Gershengorn, et al. 2004)

1) Source of islets: Islets are obtained from organ donors by the National Disease Research Interchange (NDRI). Website: http://www.ndriresource.org/NDRI_Initiatives/Pancreatic_Islets/31/

2) Lineage of cells: Primary

3) Donor information: Organ donors, male and female.

4) Experimental procedure for islet dedifferentiation (Gershengorn et al., 2004))

(1) Islets are prepared by NDRI and shipped fresh in CMRL1066 media, 1% human serum albumin, no phenopthalein. Islets are assessed by purity and viability by Dithizone (diphenylthiocarbazone) dye uptake, which allows for simultaneous viability and purity assessment. Islets are counted on 50um grids and the combination of size and staining quality contributes to overall islet quality. Our general criteria is islet size 50-250um with average ~150um diameter, > 70% purity and >90% viability.

(2) suspend 2000 islets with 10 ml of CMRL 1066 (Invitrogen, Catalog Number: 11530037) with 15% non-heat inactivated FBS in a T25 flask. Incubate at 37 C for 3 days.

(3) Change to fresh media. Incubate at 37 C for 2 days.

(4) The confluence of cells should reach 70%. Trypsinize with 0.05% trypsin-EDTA and transfer cells into a T75 flask. Incubate at 37 C for 4 days.

(5) Change to fresh media. Incubate at 37 C for 2 days.

(6) The cells should be approximately 90% confluent in the T75 flask before splitting into two T175 flasks. Incubate at 37 C for 2 days.

(7) Change to fresh media every two days and subculture the cells when confluence reaches 90%. Passage cells less than 10 times.

References:

Gershengorn MC, Hardikar AA, Wei C, Geras-Raaka E, Marcus-Samuels B, Raaka BM. 2004. Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells. *Science*. **306** (5705): 2261-4.