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Pseudoislets for diabetes research: Purifying and selective re-aggregating human islet cells V.2

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Human Islet Research Ne…

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We use this protocol and it's working

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Abstract

In vitro modeling of human islet cells for diabetes research utilizing purified and then selectively re-aggregated various combinations of human islet cells.

Note

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Guidelines

REFERENCES:

CITATION

1. Dorrell, C. et al. Isolation of major pancreatic cell types and long-term culture-initiating cells using novel human surface markers. Stem Cell Research 1, 183-194 (2008).
LINK
https://doi.org/10.1016/j.scr.2008.04.001

CITATION

2. Dorrell, C. et al. Isolation of mouse pancreatic alpha, beta, duct and acinar populations with cell surface markers. Mol Cell Endocrinol 339, 144-150 (2011).
LINK
https://dx.doi.org/10.1016%2Fj.mce.2011.04.008

CITATION

3. Liu, W. et al. Abnormal regulation of glucagon secretion by human islet alpha cells in the absence of beta cells. EBioMedicine 50, 306-316 (2019).
LINK
https://doi.org/10.1016/j.ebiom.2019.11.018

CITATION

4. Ricordi, C. et al. National Institutes of Health-Sponsored Clinical Islet Transplantation Consortium Phase 3 Trial: Manufacture of a Complex Cellular Product at Eight Processing Facilities. Diabetes 65, 3418-3428.
LINK
https://doi.org/10.2337/db16-0234

Materials

- CMRL-1066 supplemented CIT medium **Corning Catalog #**98-304-CV
- Heparin **Sagent Pharmaceuticals Catalog #**25021-400-10
- IGF-1 (Cell SciencesCat. #CRI500 **Cell Sciences Catalog #**CRI500
- fetal bovine serum **Ge Life Sciences Catalog #**SH30088.03HI
- Trypsin **Millipore Sigma Catalog #**SM-2004-C
- DPBS **Gibco - Thermo Fisher Catalog #**70011044
- Glucagon **Sigma Aldrich Catalog #**G2654
- Insulin **Dako Catalog #**A0564
- Somatostatin (SST ZYMED) **Thermo Fisher Scientific Catalog #**18-0078 (discontiued)
- ghrelin **Abcam Catalog #**ab209790
- Bovine serum albumin **Sigma Aldrich Catalog #**A6003
- PicoGreen DNA assay kits **Invitrogen - Thermo Fisher Catalog #**P7589
- Flavin adenine dinucleotide (FAD) **Sigma Aldrich Catalog #**F7378
- Propidium Iodide (PI) **Sigma Aldrich Catalog #**P4170
- Human Insulin ELISA **Mercodia Catalog #**10-1113-01
- Human Glucagon ELISA **Mercodia Catalog #**10-1271-01
- somatostatin EIA kits **Phoenix Pharmaceuticals Catalog #**EK06003

Equipment

Ultra-Low Attachment plates

NAME

Corning

BRAND

3473

SKU

https://ecatalog.corning.com/life-sciences/b2c/US/en/Microplates/Assay-Microplates/96-Well-Microplates/Costar%C2%AE-Multiple-Well-Cell-Culture-Plates/p/3473

LINK

Equipment

confocal laser-scanning microscope NAME

LSM410 TYPE

Zeiss BRAND

None SKU

https://www.olympus-lifescience.com/en/laser-scanning/fv3000/ LINK

Equipment

cell culture inserts NAME

EMD Millipore BRAND

PITP01250 SKU

https://www.emdmillipore.com/US/en/product/Millicell-Cell-Culture-Insert-12mm-polycarbonate-3.0m,MM_NF-PITP01250?ReferrerURL=https%3A%2F%2Fwww.google.com%2F LINK

1 Human islet culture and single cell preparation

Islets isolated from non-diabetic deceased human donors within 2-4 days post-isolation will be used in experiments. Islets and the dispersed cells are cultured at 37 °C with 5% CO_2 in a CMRL-1066 supplemented CIT medium (Cellgro, Cat. #98-304-CV) with 10 IU/ml Heparin (Sagent Pharmaceuticals, Cat. #25021-400-10), 1 µg/ml IGF-1 (Cell Sciences,Cat. #CRI500), and 10% fetal bovine serum (GE Life Sciences, Cat. #SH30088.03HI). For single cell preparations[1-3], islets are incubated with 0.025-0.05% trypsin (Millipore Sigma, Cat. #SM-2004-C) for approximately 6 min at 37 °C . Undispersed material is excluded using a 40 µm cell strainer. The dispersed cells are re-suspended in CMRL-1066 supplemented CIT medium with 2% FBS.

2 Fluorescence-activated cell sorting (FACS)

The dispersed cells are incubated with two monoclonal antibodies, HIC1-2B4-APC (**https://apps.ohsu.edu/research/tech-portal/technology/view/269524**) and HIC3-2D12-PE (https://apps.ohsu.edu/research/tech-portal/technology/view/3848191) at a 1:100 dilution. After incubation on ice for 30-40 minutes, the cells are washed with cold DPBS (Gibco, Cat. #70011-044), re-suspended in CMRL with 2% FBS, and sorted using the BD Influx sorter at the Flow Cytometry Core of Columbia Center for Translational Immunology (CCTI). A two-step sorting process, using the enrich mode followed by the pure sort mode, is performed to eliminate contamination from other pancreatic cell types.

3 Sorted human islet cell purity and culture

Sorted α-, β-, δ, and/or ε-cells are counted and cultured in 500 µL of CMRL-1066 with 10% FBS and 5.6 mM glucose for a period of 3-7 days in Corning Ultra-Low Attachment plates (Corning/Costar, Ithaca, NY). Each well contains at least 20,000 sorted islet cells, calculated based on the number of cells determined by flow cytometry. The cells are given 3-7 days to aggregate in culture. Intact human islets are cultured under the same conditions for control purposes.

Samples of the sorted cells are concentrated on glass slides by cytocentrifugation, fixed in 2.5% paraformaldehyde for 10 mins, and then immunofluorescence stained for human glucagon (Sigma, Cat. #G2654), insulin (Dako, Cat. #A0564), Somatostatin (SST, ZYMED, Cat. #18-0078) and ghrelin (Abcam, Cat. #ab209790) to confirm purity. Secondary antibodies conjugated with Dylight-dyes (Jackson ImmunoResearch) are used for fluorescence detection. Digital images of fluorescence-labeled cells are acquired using a Zeiss fluorescence axial microscope attached with a digital camera or with a Zeiss LSM410 confocal laser-scanning microscope.

4 In vitro glucose challenge

Duplicate or triplicate samples from each donor are tested for each treatment condition. Cells from each donor are used as their own controls when testing and comparing function under the various treatment conditions.

After the 3-7 day culture, the cell aggregates and control intact islets are subjected to glucose challenge using a static incubation approach described for intact islets[4] with modifications. The cell aggregates or islets are carefully transferred into individual cell culture inserts with a pore size of 3 µm (EDM Millipore, Cat. #PITP01250) in 24-well plates (Non-tissue culture treated, Corning). The cells are first equilibrated in 2.0 mM glucose-containing HEPES-balanced Krebs-Ringer bicarbonate buffer (KRB) with 2 mg/ml Bovine serum albumin (Sigma-Aldrich, Cat. #A6003,) at 37 °C , 5% $CO_2$for 1 hr. Following this, the inserts are removed from the wells, drained of any residual media and transferred to new wells containing KRB with 2.0 mM glucose and incubated at 37 °C for one hour. Afterwards, the inserts are transferred to new wells containing KRB supplemented with various concentrations of glucose and growth factors or compounds (e.g., Glibenclamide) for another hour. The cells used in the high-to-low glucose challenge are first incubated in KRB with high concentration glucose for one hour and then transferred to KRB containing 2.0 mM glucose for another hour. The supernatants from each of the one hour-incubations of the two step-glucose challenge assay are collected for islet hormone measurements. The cells are lysed for cell number quantification at the end of the experiment using the PicoGreen DNA assay kits (Invitrogen, Cat. #P7589) according to the manufacturer's protocol. In addition, subgroups of cells are also evaluated for viability using FAD (Sigma, Cat. #F7378) and PI (Sigma, Cat. #P4170) staining, and for glucagon content assessment by acid ethanol extraction (1.5% HCL in 75% ethanol) followed by freezing and neutralization steps prior to glucagon concentration measurement.

5 Islet endocrine hormone measurements

The supernatant collected are analyzed for insulin and glucagon concentrations using the human insulin and glucagon ELISA kits (Mercodia, Cat. #10-1113-01 and #10-1271-01) and somatostatin EIA kits (Phoenix Pharmaceuticals, Cat. #EK06003) according to the manufacturers' instructions. The hormone released by each group of cells was expressed as the ratio of the hormone released during the second step incubation divided by that released in the first step incubation of the two-step glucose challenge assay, as described above. The ratio represents the function of cells in each sample in a cell-number independent manner. In addition, in order to compare the absolute amount of hormone secretion by a given number of cells under different experimental conditions, glucagon or insulin release was standardized to cellular DNA content measured as described above.