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Isolating Placental Organoids from Full-term human placental tissue

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Reagents needed:

Wash buffer:
HAMS/F12 medium (Thermo Fisher, 11765054) supplemented with 1x pen/strep

Digestion stop solution:
20% FBS/wash medium

0.2% Trypsin-250/0.02% EDTA/PBS solution:
0.3g glucose, 12g NaCl, 0.3g KCl, 1.725g disodium hydrogen orthophosphate, 0.3g potassium dihydrogen orthophosphate, 2g trypsin (Alfa Aesar, J63993-09), 0.2g EDTA (Sigma-Aldrich, E9884-100G) made up in 1L of water. Filter, aliquot, and store for up to 6mo at -20°C.

Collagenase V digest solution:
1.0 mg/mL Collagenase V (Sigma-Aldrich, C9263-100MG) in Ham's F12/K with 10% FBS. Make fresh for each use.

Matrigel stocks:
Stocks should be thawed overnight at 4°C.

Tissue dissection and digestion

Pre-warm 0.2% Trypsin/0.02% EDTA and Collagenase V solutions at 37°C

1. Dissect placental chorionic villi. Carefully remove decidua.
2. Generate very small fragments of tissue.
3. Place tissue fragments in glass bottle containing wash buffer and a stir bar and wash extensively on a magnetic stirrer (VWR, 76447-030) at room temperature as much as possible (at least 5 times). Continue to wash until wash buffer is clear.
4. Allow tissue to settle to bottom of glass bottom by gravity and then remove wash buffer using a vacuum aspirator.
5. Pour 25-75mL (depending on quantity of fragments) of pre-warmed 0.2% Trypsin/0.02% EDTA solution into the same glass bottle containing a small stir bar. Place bottle on a heated (37°C) stir plate with gentle stirring (set at ~ 80 rpm). Incubate for 8min.
6. Filter the suspension using muslin gauze placed over a funnel. Add digestion stop solution to neutralize trypsin. *Save the flow through as you will use this in a subsequent step*. *Be sure to save the undigested tissue, which will be used in the next step.*
7. Retrieve the undigested tissue from the muslin gauze and place in 12mL of collagenase V solution in a glass bottle containing a stir bar. Place the bottle on a heated stir plate and stir with gentle agitation (~ 80 rpm) for 8min.
8. Remove the glass bottle from the stir plate and manually disrupt tissue using a narrow orifice 10mL serological pipette (VWR, 89130-898). Forcefully pipette up and down ~10 times to break up tissue prior to filtration.

9. Filter the suspension as described in step 6. Remaining undigested tissue on the gauze can be discarded.
10. Pool the cells collected in steps 6 and 9.
11. Centrifuge at 400g for 5min.
12. Resuspend the pellet in 5mL of Advanced DMEM/F12 medium (Life Technologies 12634-010) and transfer to a 15mL conical.
13. Centrifuge at 600g for 5-6min to pellet.
14. Carefully remove as much of the supernatant as possible. *Do not disrupt the pellet*. It is not recommended to use vacuum aspiration for this step being careful not to disrupt the pellet.
15. Resuspend the pellet with pre-thawed Matrigel (Corning 356231) using blunt 200 µl pipette tips (Fisher 02-707-134). The volume will depend on how many wells will be plated. 40uL of Matrigel is used per well. Use pre-chilled large orifice pipette tips to prevent Matrigel polymerization due to temperature.
16. Carefully dispense 40uL of cells/Matrigel to the middle of each well of a pre-warmed 24-well plate to create a "dome".
17. Carefully place the plate in the 37°C incubator for 3min to allow for pre-polymerization.
18. Flip the plate upside down to disperse cells throughout the Matrigel dome. Incubate for 8min.
19. Add 500uL of growth medium containing ROCK inhibitor Y-27632 (to prevent stem cell death) and culture for 72-96h in this medium. After this time, add fresh medium without Y-27632.

Media recipe:

Trophoblast organoid medium (TOM)

The following is the recipe of preparing 50 mL TOMs (# 6, 4th version)

Ingredient	Volume(µl)	Final Concentration
100 × N2 (Life Technologies, 17502-048(	500	1×
50 × B27 (Life Technologies, 17504-044)	1000	1×
500 × Primocin (InvivoGen, ant-pm-1)	100	100 µg/ml
80 × NAC (Sigma, A9165)	625	1.25 mM
100 × L-glutamine (Life Technologies, 35050-061)	500	2 mM
10000 × A83-01 (Tocris, 2939)	5	500 nM
10000 × CHIR99021 (Tocris, 4423)	5	1.5 µM
2000 × recombinant hEGF (Gibco, PHG0314)	25	50 ng/ml
2000 × recombinant R-spondin1 (R & D systems, 4645-RS-100)	25	80 ng/ml
2000 × recombinant hFGF2 (Peprotech, 100-18C)	25	100 ng/ml
2000 × recombinant hHGF (Peprotech, 100-39)	25	50 ng/ml
100 × Nicotinamide (NTM) (Sigma, N0636-100G	500	10 mM

500 × Y-27632 (Sigma, Y0503-1MG)	250	5 μM ↑
2000 × PGE2 (R & D systems, 22-961-0)	25	2.5 μM
FBS (heat inactivated) (Cytiva HyClone, SH30070.03(	5 mL	10% (vol/vol)
Advanced DMEM/F12 (Life Technologies, 12634-010)	Adjust to 50 mL	N/A

Annotation: First add about 35 mL Advanced DMEM/F12 to the 50 mL centrifuge tube, then add the above supplements, adjust the final volume to 50 mL with Advanced DMEM/F12. Use the full medium within 1 month. The red highlighted are supplemented components post optimization for full-term placental tissue.