DEC 18, 2023

OPEN ACCESS

Protocol Citation: vfarmer 2023. Isolation of Trophoblast Cells from Placenta. **protocols.io** https://protocols.io/view/isolation-of-trophoblast-cells-from-placenta-c6eyzbfw

Protocol status: Working
We use this protocol and it's working

Created: Dec 14, 2023

Last Modified: Dec 18, 2023

PROTOCOL integer ID: 92344

Isolation of Trophoblast Cells from Placenta

vfarmer[1]

[1]Duke University

vfarmer

ABSTRACT

Protocol to derive trophoblast stem cells from placenta and culture them to form trophoblast organoids

Preparations

1 0 °C the following items (and preferably have extras on had if needed):
- 2 100mL bottles
- 2 small stir bars
- 1 large funnel
- cheese cloth cut into squares that are large enough to sit in the funnel
- dissection scissors

2 Equipment and Reagents Needed:
- stir plate that heats. alternatively could use a shaking water bath
- scalpels for dissection
- Matrigel, thawed
- Red Blood Cell Lysis Buffer (invitrogen, 00-4333-57)
- blunt P200 tips pre-chilled 4 °C
- 24-well plate warmed to 37 °C

3 Prepare the following Solutions and place all at 37 °C

The components needed to make all of the solutions below are:
- F12-K
- PennStrep
- 2.5% Trypsin
- Collegenase
- FBS

3.1 **Wash Solution**: F12K + PennStrep

500 mL bottle of F12-K + 5 mL PennStrep

- have P/S aliquoted in 5 ml aliquots and only thaw when needed
- keep P/S aliquots in -20C
- I typically use ~400 mL of Wash Solution. No reason to need to make fresh each time, can use leftover as long as it is only a few months old

3.2 **Trypsin Solution**: 0.2% Trypsin

75 mL of 0.2% Trypsin needed
Note: volume can be down to 25 mL, with the volume depending on the amount of starting tissue. I like to consistently use 75 mL.

6 mL 2.5% trypsin + 69 mL PBS = 75 mL 0.2% trypsin

3.3 **Collagenase Solution**: 1 mg/ml collagenase in Wash Solution

25 mL of collagenase

Dissolve 25 mg in 25 mL F12-K + P/S (Wash Solution)

3.4 **Stop Solution**: Final concentration of 10% FBS to inhibit Trypsin

Thaw FBS and use as needed. When using 75 mL of trypsin, I typically add 10 mL of 100% FBS to yield a final of just over 10% FBS

3.5 **TOM**: will need for the final plating

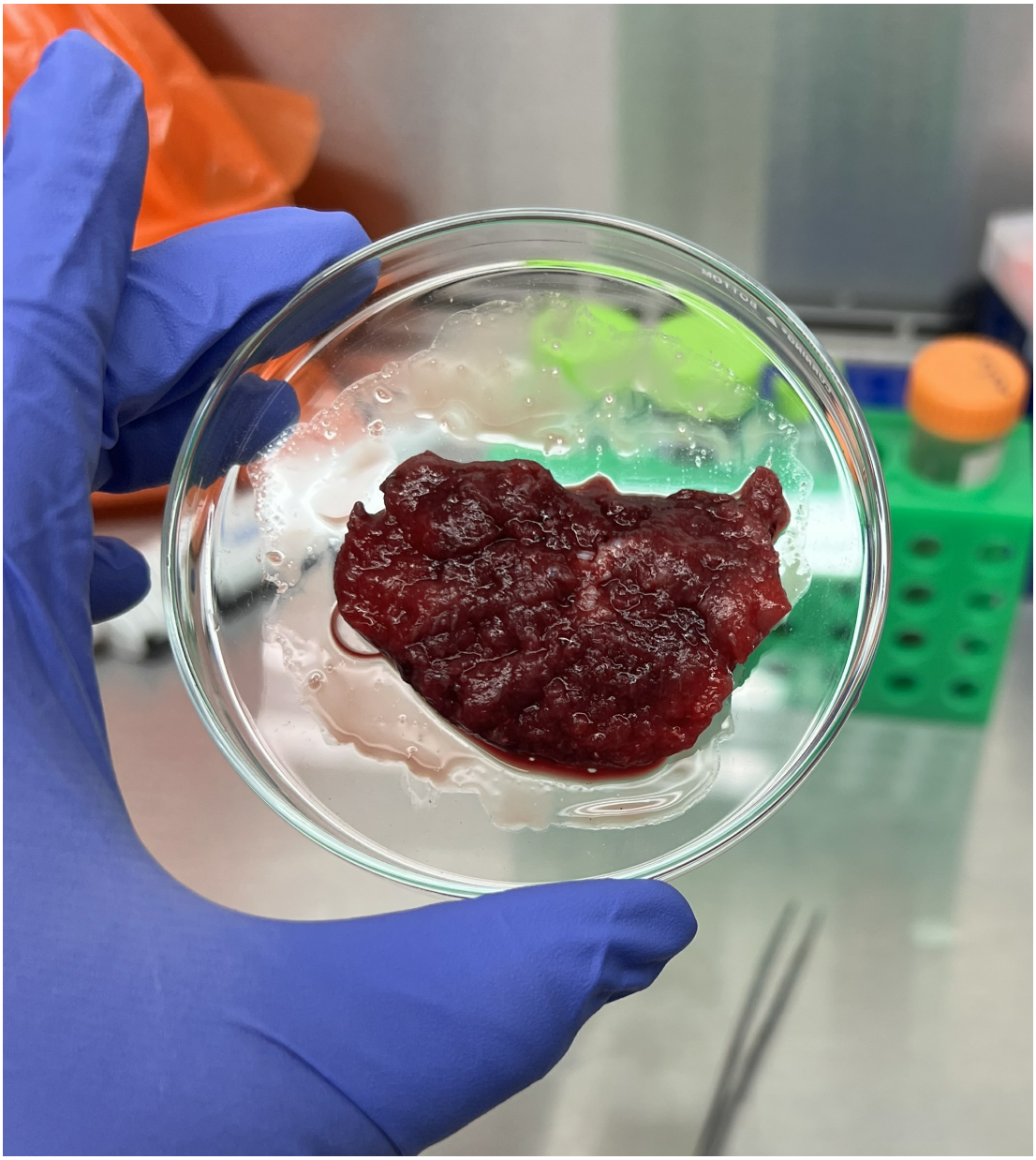
I typically plate 6 wells, therefore 3 mL of TOM is needed.

Will need to supplement TOM with Y-27632 so the final concentration is 10 µM (add 1 µl of Y-27632 for every 500 µl of TOM).

Isolation Protocol

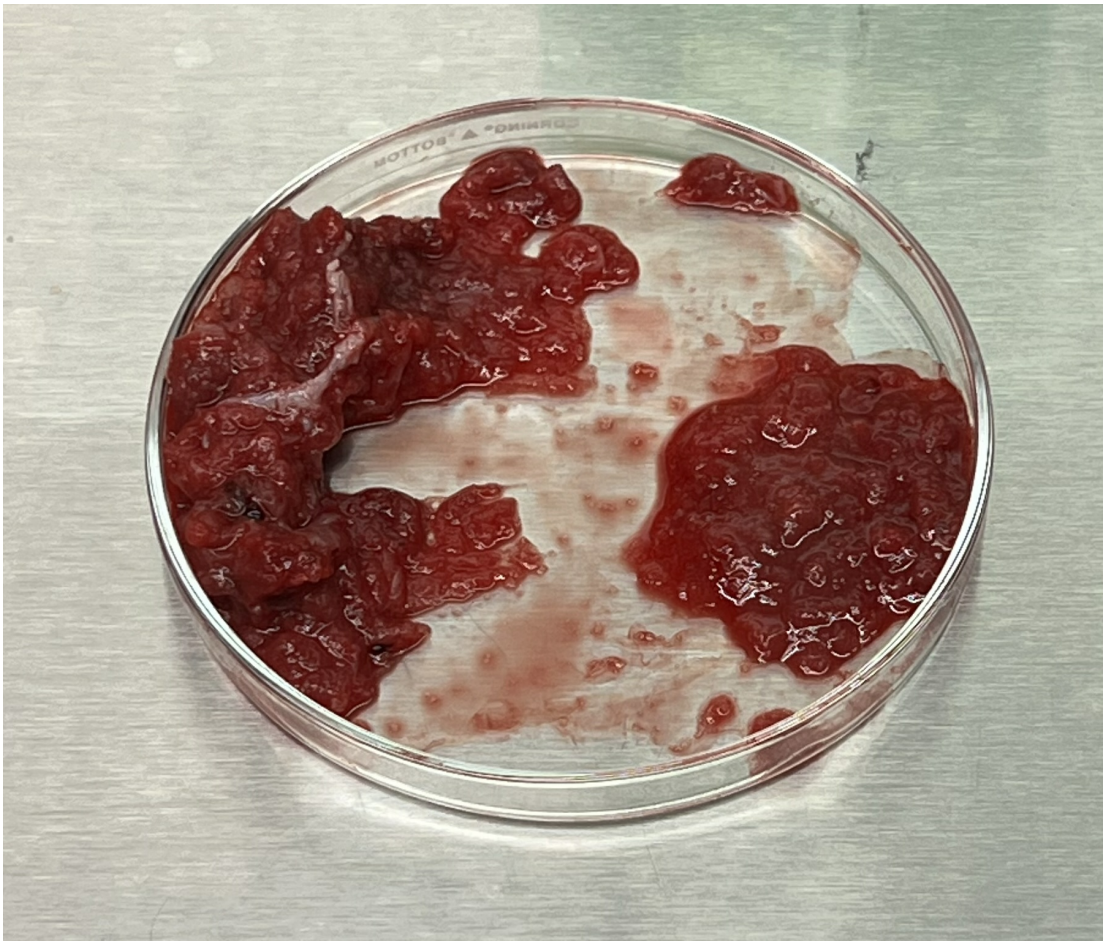
39m

4 Cut out a lobule from the placenta. Carefully cut off the decidua to reveal chorionic villi, see image below:



Decidua removed, villi facing up

5 Using a scalpel, generate very small fragments of tissue by scraping tissue off of the villus trees. The villus tress will be white veins running throughout the tissue. The smaller the tissue fragments, the more successful the isolation. Tissue should look like the tissue on the right side of the image below:



Tissue all cut up. Tissue on left will not be used, tissue on right will be used for the rest of the protocol.

6 Place the tissue fragments into a 100 ml glass bottle and wash extensively with **Wash Buffer**.

6.1 Add 50 mL of **Wash Buffer,** put cap on bottle and swirl bottle around ~5 times to wash tissue. Place bottle down and let tissue settle to the bottom of the bottle. Remove wash buffer using vacuum.

Note: Some tissue can get aspirated up with the tissue, depending on how well the tissue settles to the bottom of bottle. I have found that not introducing air bubbles while swirling helps increase the chance of the tissue sedimenting to the bottom of the bottle. As the wash steps go on, sedimentation gets worse and worse. Could think about centrifuging, but it hasn't been an issue yet for me.

6.2 Repeat this at least 5 times! Wash Buffer will turn less dark pink/red throughout washes.

6.3 When the Wash Buffer does not change from its light pink color, tissue is thoroughly washed.

7 Add 75 mL of Trypsin Solution and two small stir bars to the glass bottle containing the washed tissue. Place bottle on 40 °C stir plate and incubate for 00:08:00 with gentle agitation. 8m

Note: I use 125 rpm on my stir plate. I use two small stir bars because I have found them bumping into each other helps the tissue disperse and not just stay in one clump.

7.1 Add FBS to a final concentration of about 10% to inactivate Trypsin. Add 8 mL of FBS.

Set bottle aside, will filter Collegenase/Tissue solution into this bottle later.

8 Place funnel on second glass bottle and place cheese cloth in funnel. Filter Trypsin/Tissue solution through cheese cloth.

Note: Don't squeeze things through the cheese cloth; I have found an increase in the amount of debris when I do this

9 Gently scrape off tissue on cheese cloth and place it back in the same glass bottle with the stir bars. A 8m 25 mL of **Collegenase Solution** and incubate for 00:08:00 with gentle agitation on a 40 °C stir plate.

10 Place funnel in the glass bottle containing the filtered cells and place new cheese cloth in funnel.

10.1 Remove bottle from stir plate and manually disrupt the tissue using a narrow orface 10 mL serological pipette. Forcefully pipette up and down ~10 times to break up the tissue prior to filtering through cheese cloth.

10.2 Filter Collegenase/Tissue solution through cheese cloth into glass bottle containing the previous Trypsin/Tissue filtrate.

11 Centrifuge filtrate at 300 rcf, Room temperature, 00:04:00 . I typically have 100 ml of filtrate and spilt between two 50 ml conicals.

12 Remove supernatant using the vacuum (carefully, and don't touch pellet!). Resuspend pellets in 10 mL of F12-K and transfer to a 15 ml conical. I will resuspend both pellets in 5 mL, then wash each conical with the remaining 5 mL.

13 Centrifuge at 300 rcf, Room temperature, 00:04:00 4m

14 Carefully remove supernatant using serological pipette. Be careful not to disturb pellet!

15 Resuspend pellet in 1 mL of Red Blood Cell Lysis Buffer. Incubate at Room temperature for 5m 00:05:00.

Note: This step is not essential but it does significantly help remove unwanted cells

16 Centrifuge at 300 rcf, Room temperature, 00:04:00 4m

17 Carefully remove supernatant using P1000, then use P200 to remove any excess media. Place conical in ice bucket.

18 Resuspend the pellet with 240 µL of pre-thawed Matrigel using a pre-cooled blunt 200 µL pipette tip (Fisher 02-707-134).

19 Carefully dispense 40 µL of Matrigel-organoid suspension into a pre-warmed 24-well plate using a cold pipette tip.

Notes: I usually set the pipette to 35 µl (there will be lose of some Matrigel/cell mixture). Slowly and carefully lift up the pipette as dispensing the Matrigel into the well to form a dome. Do not push pipette tip fully down as this will introduce air-bubbles.

20 Place the 24-well plate in 37 °C incubator for 00:02:00 to allow Matrigel to pre-polymerize 2m

21 Flip the plate over and incubate for an additional 00:08:00 to fully polymerize and evenly distribu 8m the organoid fragments throughout the Matrigel

22 During the polarization process, prepare a stock of TOM with Y-27632 (final concentration 10 µM; add 1 µl of Y-27632 per 500 µl of TOM).

Note: Need 500 µl of medium per well

23 Cover the polymerized Matrigel domes with 500 µl TOM per well and culture them in a 37 °C humidified 5% CO2 incubator.

Note: Fill surrounding wells with 1 ml of PBS to help decrease evaporation of TOM

24 Keep an eye on the cells over time and replace media every 2-3 days. Keep extra Y-27632 (final of 10 µM) for the first 3 media changes.

Notes: There will be so much debris, it will be impossible to see single cells. Debris and other cells will die overtime and clear organoids will begin to emerge after about 2-3 weeks.