Non-Enzymatic Generation of Placenta Single Cells from Third Trimester Human Placenta.

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Abstract

The placenta is a heterogeneous and complex organ with multiple cell types, posing a challenge for the field of maternal-fetal medicine to implement single-cell technologies for a deeper characterization of this essential organ. Several protocols use enzymes to digest the tissue and generate single cell suspension, but this approach has several shortcomings including the loss and reduced viability of cells. In this study, we describe a non-enzymatic approach to generate single cell suspensions from placental tissue with high yield and viability for single cell RNA sequencing.

Protocol.

Human subjects and tissue samples.

This protocol has been successfully utilized for the preparation of single-cell and single nuclei suspensions from freshly collected placental sections. Tissues were obtained from women with a normal pregnancy term with labor.

Donors were recruited into the institutionally approved African Materno-Fetal System during Pregnancy at Single-cell Resolution research protocol of the Barau Dikko Teaching Hospital

Stage 1. Sampling Procedure.

1. Take photographs of the maternal and fetal surfaces of the whole placenta.
2. ensure the placenta is placed on a sterile surface.
3. Collect placenta sections spanning the cross-section of the maternal and fetal side of the placenta.
4. Eppendorf tubes should be labelled appropriately to represent regions of the placenta sampled.
5. Add 1ml of MACs tissue storage buffer in labelled Eppendorf tubes.
6. Keep tubes on ice.

Stage 2. Collection and Transport of human placenta biopsies

1. Carefully and aseptically collect 10mg-1g placenta biopsies into 1.5ml Eppendorf tubes with 1ml of cold macs tissue storage solution.
2. Ensure that all placental biopsies are completely immersed in storage buffer solution and other tissue biopsies are well placed in in the empty Eppendorf tubes.
3. Keep all samples on ice.

Proceed to stage 3 as soon as possible within one hour.

Stage 3. Placenta tissue dissociation on a gentle MACs dissociator

1. Transfer exactly 10mg of placenta tissue into a well labelled gentle MACs C- tube containing 3ml MACs running buffer.
2. Fix each C-tube on the gentle macs dissociator using tube heaters.
3. Set the dissociator program to h_cord_01
4. dissociate on the gentle MACs dissociator using the program h_cord_01 at 553 rotation per round (rpr) for 30 seconds.

Note on h_cord_01 program installation on the gentle macs dissociator.

Proceed to stage 4 within 1minute, 30 seconds.

Stage 4. Enrichment of placenta single cells.

1. Use a 1000µl pipette and transfer each placenta single cell and tissue mixture into well labelled 1.5ml Eppendorf tubes (multiple tubes should be used to accommodate all the mixture from the MACs C- tube) and centrifuge at 300g for 10 minutes at 4^{O}C.
2. Using a wide bore pipette carefully remove supernatant without disturbing the pellet.
3. Discard the pellet.
4. Repeat steps 2 and 3.
5. Pool supernatant together in a 2ml Eppendorf tube and centrifuge at 500g for 5min at 4^{O}C.
6. Remove entire supernatant leaving approximately 20ul.
7. Reconstitute placenta single cell pellet in 20ul supernatant.

Maintain cells on ice and proceed to stage 5 immediately

Stage 5. Placenta single cell number and viability assessment

1. Take approximately 10µl of each supernatant recovered from centrifugation process and drop it into the well on the counting chamber slide.
2. Insert the slide into the countess III automated cell counter.
3. Press count on the machine
4. Cells are ready for downstream application.

S/N	EQUIPMENT/CONSUMMABLES	COMPANY
1.	Styrofoam box with insulated container	Uline
2.	1.5ml microcentrifuge tubes	Eppendorf
3.	Ice making machine	Kojak
4.	Weighing Balance	Halomedicals Systems Limited
5.	Gentle MACs Dissociator	Miltenyi Biotech
6.	Gentle MACs C- tubes	Miltenyi Biotech
7.	Countess III Chamber slides	Thermo Fisher Scientific
8.	Countess III Automated Cell counter	Thermo Fisher Scientific
9.	100- 1000µl Micro pipette	Agros 240-21 Omega 8
	0.1- 10µl Micro pipette	Agros 240-21 Omega 8
10.	Refrigerated Micro Centrifuge	Eppendorf
11.	100µl Pippete tips	Argos technologis
12.	10µl Pippete tips	Argos technologis
13.	1000µl Pippete tips	

REAGENTS

S/N	REAGENTS	COMPANY
1.	MACs Tissue Storage solution	Miltenyi Biotech
2.	MACs running buffer	Miltenyi Biotech

Meta data

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Samples	Gestational age	Time of delivery	Placenta shape	Umblical cord diameter	Weight of the placenta	Health status of the donors	Age of the donors	Baby's BP
Placenta 1	40 weeks	10:45 am	Irregular	54X0.5	500g	Normal	32 years	60/40
Placenta 2	44 weeks	11.55 am	discoid	52X0.5	440g	Normal	25 years	70/50