APPLICATION NOTE

Isolation of cytotrophoblast cells by density gradient centrifugation

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Abstract

Most human placental functions are carried out by the syncytiotrophoblast, a multinuclear layer formed by intercellular fusion of underlying cytotrophoblast cells. The endocrine and immunological functions of syncytiotrophoblasts and the ion exchange between the mother and her fetus are studied by using cytotrophoblast cell culture as a model system.

This note shows the procedure for isolation and purification of cytotrophoblast cells from human placenta using the Thermo Scientific™ 4-liter refrigerated general purpose centrifuge. An efficient and reliable isolation of cytotrophoblast cells was achieved.

Introduction

The human placenta is a fetal organ which supports and maintains the pregnancy. It plays a crucial role in protection, nutrition and respiration of the fetus. Most human placental functions are carried out by

Figure 1. Thermo Scientific 4-liter refrigerated general purpose centrifuge.

the syncytiotrophoblast, a multinuclear layer formed by intercellular fusion of underlying cytotrophoblast cells. The endocrine and immunological functions of syncytiotrophoblasts and the ion exchange between the mother and her fetus are studied by using cytotrophoblast cell culture.

Cytotrophoblast cells are purified following the protocol of Kliman et al. with modifications by Petroff et al. and Nikitina et al. The method involves Trypsin-DNase digestion, followed by density gradient centrifugation to remove cellular debris, red blood cells and polymorphonuclear cells.

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Materials and methods

Table 1. Equipment

Equipment	Supplier	Cat. No.
4-liter refrigerated general purpose centrifuge: Thermo Scientific™ Heraeus™ Multifuge™ X3R	Thermo Fisher Scientific	75004515 75004516
4-liter refrigerated general purpose centrifuge: Thermo Scientific™ Sorvall™ Legend™ XTR	Thermo Fisher Scientific	75004520 75004521 75004522 75004523
Rotor: TX-750	Thermo Fisher Scientific	75003180
Buckets	Thermo Fisher Scientific	75003608
Adapter for 50 mL tubes	Thermo Fisher Scientific	75003638
Adapter for 15 mL tubes	Thermo Fisher Scientific	75003639
Flow Cytometry: BD LSR II SORP	Becton Dickenson	–
Flow Cytometry: FlowJo™	FlowJo	–

Table 2. Centrifugation conditions

	RCF (x g)	Time (min)	Temperature (°C)	Acceleration/deceleration
Tissue preparation	1,000	15	10	9/9
Density gradient centrifugation	1,200	20	22	6/0

Table 3. Reagents

Growth media composition	Supplier	Cat. No.
0.25% Trypsin	Invitrogen™	15090/046
DNase I 300 U/mL	Sigma™	D5025-150KU
Fetal calf serum (FCS)	Seraglob	S40500
Medium: DMEM 1x GlutaMax-I	Gibco™	32430
CMF-Hank solution: Ca/Mg free HBSS (1x HBSS + 25 mM HEPES ph = 7.4) HBSS 10x = 53.6 mM KCl, 4.4 mM KH_2PO_4, 1.37 M NaCl, 3.37 mM Na_2HPO_4, 55.5 mM D-Glucose	–	–
Percoll	Sigma	P4937-500ML
Mouse anti-human cytokeratin-7 antibody	Dako™	M7018
Goat anti-mouse Alexa Fluor 488	Invitrogen	A-11001
Anti-Anti 100	Gibco	15240

Tissue samples

Placental tissues were received from the Lindenhofspitalgruppe, Bern, Switzerland, with prior consent from pregnant women. Ethical approval was achieved by the local institutional review board.

Isolation and cultivation of cytotrophoblast cells

a. Tissue preparation

After collection, the tissue was washed 3–4 times in 0.9% NaCl. Minced tissue was added to an Erlenmeyer flask and subjected to sequential enzymatic digestions with 0.25% Trypsin and DNase I 300 U/mL. After incubation for 20 min at 37 °C the tissue was filtered through a metallic mesh (180 µm). The rest of the tissue was digested and incubated as described above. Filtrated digestion suspension was added to 1.5 mL fetal calf serum in a 15 mL tube and centrifuged at 1000 x g, 15 min and 10 °C. The supernatant was discarded and the pellet was resuspended in 2 mL medium. The cells were collected in 50 mL tubes. The procedure described above was repeated 3 more times. Then the cell suspension was filtered through a nylon cell strainer (100 µm). 50 mL medium was added and the suspension was centrifuged at 1000 rpm and 10 min. The supernatant was discarded and the pellet was resuspended in CMF-Hank solution.

b. Density gradient centrifugation

The cells collected during the digestions were purified on a noncontinuous Percoll gradient. For gradient dilutions 90% Percoll was used. The gradient was built up in 50 mL tubes, beginning with highest (70%) to the lowest (5%) in 5% steps. 3 mL cell-suspension was applied to the top of the Percoll gradient. The gradient was centrifuged at 1200 x g, for 20 min at 22 °C. The acceleration profile 6 and deceleration profile 0 were set. After centrifugation, the cell migrated in the layer corresponding to 1.048 g/mL–1.060 g/mL density was collected.

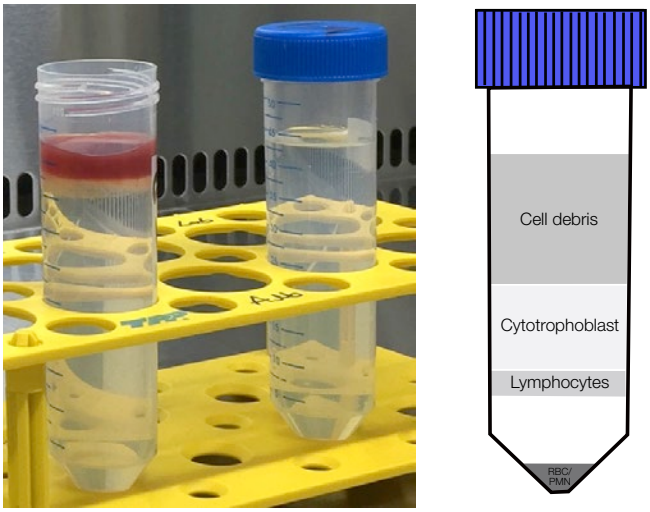

Figure 2. Left: Cell-suspension was applied to the top of the Percoll gradient; Percoll gradient, starting from 70% to 5 %. **Right:** Schematic overview of Percoll gradient and cell types after centrifugation.

Flow cytometry

Isolated cytotrophoblast cells were fixed and permeabilized in 100% methanol (ice cold) for 10 min on ice. Cell were washed in DPBS and resuspended in DPBS + 10% FBS. Anti-cytokeratin 7 and 18 antibodies were used as cytotrophoblast markers.

Cell suspension was transferred into flow cytometer tubes and analysis was performed on a BD LSRII SORP (Becton Dickinson) flow cytometer and data were analyzed using FlowJo.

Results

The purity of isolated cytotrophoblast cells was 92–95% as assessed by cytokeratin-7 expression using flow cytometry. Contamination by other cells was minimized.

Figure 3. Density gradient centrifugation. Cytotrophoblast cells are located in the cell layer corresponding to 1.048 g/mL–1.060 g/mL density.

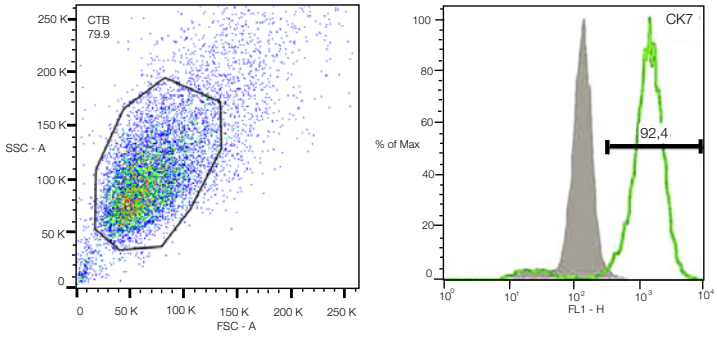

Figure 4. Characterization of isolated cytotrophoblast cells was performed using flow cytometry. Primary cytotrophoblast cell populations were positive for the trophoblast cell marker CK7.

Conclusion

This application note demonstrates an efficient and reliable isolation of cytotrophoblast cells using the Thermo Scientific™ 4-liter refrigerated general purpose centrifuge with the Thermo Scientific™ TX-750 rotor. Excellent purity (92–95%) of cells are obtained for subsequent analysis.

References

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