Isolation of Trophectoderm and Inner Cell Mass from Bovine Blastocysts using Magnetic Activated Cell Sorting

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The first distinct lineage differentiation in the mammalian embryo occurs at the blastocyst stage when blastomeres are segregated into inner cell mass (ICM) or trophectoderm (TE). Obtaining purified TE or ICM can be useful for understanding regulation of early development and differentiation. Although several methods have been reported to separate TE and ICM, e.g., immunosurgery, mechanical dissection using a micromanipulator, or manual selection following trypsinization, limitations exist with these methods. Here, we describe a simple and effective method to sort cells of the blastocyst using magnetic activated cell sorting (MACS) following disaggregation of the blastocyst into single cells using trypsin.

The method has been published: Ozawa, M., and Hansen, P.J. (2011) A novel method for purification of inner cell mass and trophectoderm cells from blastocysts using magnetic activated cell sorting. *Fertil. Steril.* 95, 799-802.

A schematic illustration of the technique is presented in Figure 1.

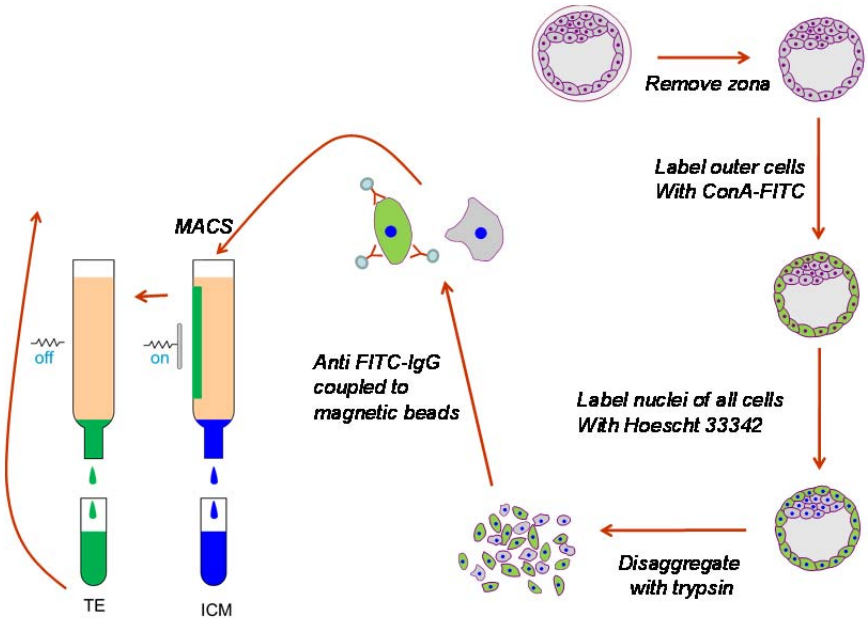

Figure 1. Schematic illustration of the technique to separate TE from ICM using MACS. The outer TE cells are labeled with ConA-FITC. Following aggregation, TE cells are labeled with anti-FITC coupled to magnetic beads and cells are then separated by MACS.

Materials and Reagents

- Acidic Tyrode's solution (cat. no. MR-044-D, Millipore)
- Dulbecco's PBS: (DPBS, cat. no. 14190, Invitrogen)
- MACS buffer (DPBS containing 0.5% (w/v) BSA and 2 mM EDTA, pH 7.2)
- Concanavalin A (ConA) conjugated to FITC (cat. no. C-7642, Sigma)
- Hoechst 33342 (cat. no. B2261, Sigma-Aldrich)
- 0.05% (w/v) Trypsin-EDTA solution (cat. no. 25300-054, Invitrogen)
- Fetal bovine serum (Atlanta Biologicals)
- Cell strainer (cat. no. 352360, BD Biosciences)
- MACS separation column (cat. no. 130-042-202, Miltenyi Biotec)
- Magnetic board (cat. no. FMS-1000, Spherotech, Lake Forest, IL, USA)

Methods

1. Remove the zona pellucida from blastocysts by short exposure to acidic Tyrode's solution (pronase treatment also works, click here for the protocol.
2. Wash three times in MACS buffer.
3. Incubate the zona-free blastocysts with concanavalin A (ConA) conjugated to FITC (1 mg/ml in MACS buffer) for 10 min in the dark to label the outer layer of the blastocyst.
4. Wash three times in MACS buffer (**Note**: If you want to observe cells before or after MACS, stain the zona-free blastocysts at this step with 1 μg/ml Hoechst 33342 in MACS buffer for 3 min to label nuclei of all blastomeres. Wash three times after Hoechst 33342 staining. Alternatively, stain an aliquot of cells at the end of the MACS procedure with Hoescht so as to avoid exposing cells used for DNA/RNA extraction with Hoescht).
5. Incubate the zona-free blastocysts in DPBS containing 1 mM EDTA for 5 min.
6. Transfer zona-free blastocysts to trypsin-EDTA solution and incubate for 15 min at 38.5°C.
7. Wash three times with a wide bore pipette in MACS buffer.
8. Transfer the zona-free blastocysts to 100 µl drop of MACS buffer and disaggregate into single blastomeres by vortexing for 10 seconds, repeated three times.
9. Transfer the drop containing the disaggregated blastomeres into a 1.5 ml tube and mix with 500 µl DPBS containing 1 mM EDTA and 10% (v/v) fetal bovine serum to inactivate trypsin.
10. Wash twice in MACS buffer by centrifugation (500 x g, 5 min).
11. Reconstitute the disaggregated blastomeres with 500 µl MACS buffer.
12. Eliminate large clusters of cells by passing the suspension over a cell strainer, and collect the single blastomeres that passed through the strainer.
13. Wash the blastomeres once using MACS buffer by centrifugation (500 x g, 5 min) and resuspend the blastomeres in 110 µl MACS buffer.

14. Incubate the blastomeres with 10 µl magnetic microbeads conjugated to mouse anti-FITC IgG1 for 15 min on ice in the dark.
15. Wash blastomeres three times with MACS buffer by centrifugation (500 x g, 5 min) and resuspend in 500 µl MACS buffer.
16. Condition the MACS column by adding 500 µl MACS buffer.
17. Load the blastomeres contained solution into a MACS separation column attached to a magnetic board.
18. Wash the column twice with MACS buffer (500 µl each) to obtain the FITC negative blastomere fraction (equivalent to ICM). .
19. Detach the column from the magnetic board and wash three times using MACS buffer (500 µl each) to recover the FITC positive fraction (equivalent to TE).

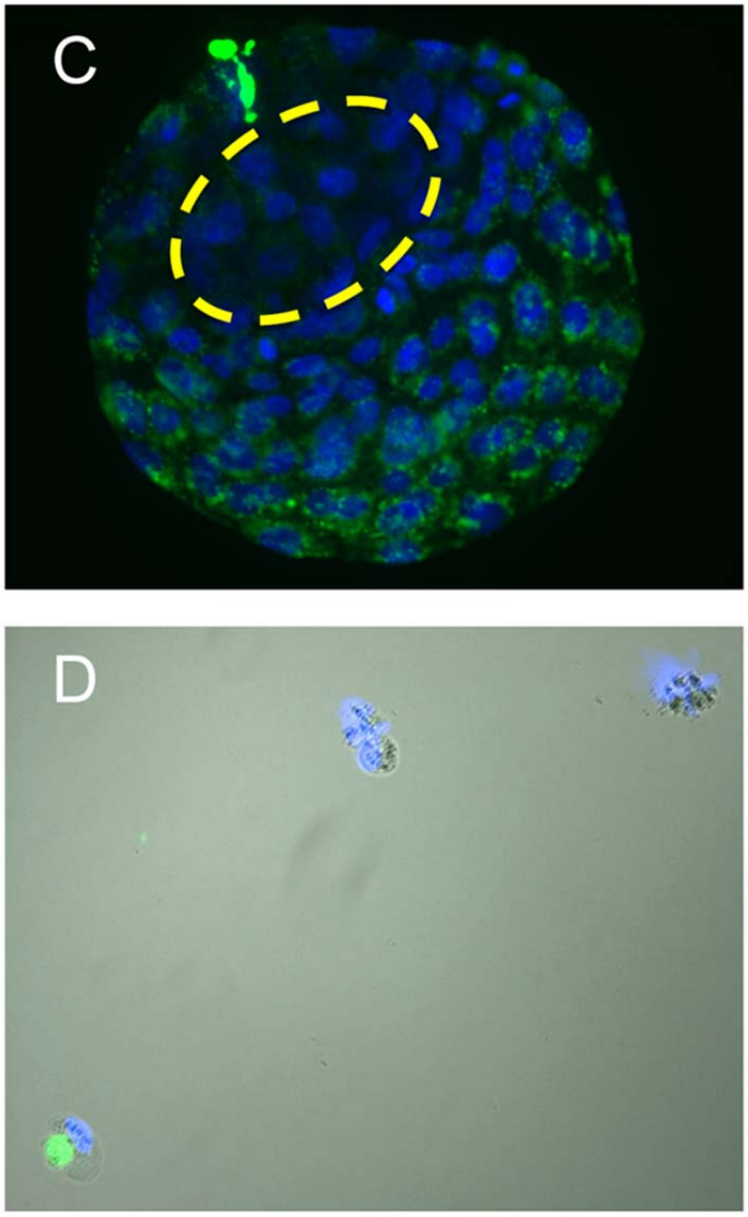

Figure 2. Differential labeling of inner cell mass and trophectoderm. Panels C represent labeling of a representative blastocyst with ConA-FITC (green) and Hoescht 33342 (blue). Panel D shows individual blastomeres of the blastocyst after disaggregation by trypsinization followed by repeating pipetting using a finely drawn flame-polished mouth micropipette. Note that one cell, dual-labelled with ConA-FITC and Hoescht 33342, is a trophoblast cell and the other cells, labelled with Hoescht 333442 only, are inner cell mass cells. From Ozawa and Hansen (2011).

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