Mouse Endothelial Cells

Simple, fast and efficient isolation of mouse endothelial cells

Isolated cells are pure and free of contaminating fibroblasts

Introduction

Endothelial cells (EC) form the lining of the entire vascular system, from the heart to the smallest capillary and control the passage of materials and leucocytes into and out of the bloodstream. A pure population of cells, free from contaminating fibroblasts is required to study EC. Murine tissue is digested to obtain a single cell suspension and Dynabeads® coated with an anti-murine EC antibody are added directly to the cell suspension to capture EC. The bead-bound EC can be collected by a magnet, Dynal MPC®, to give a pure endothelial cell fraction.

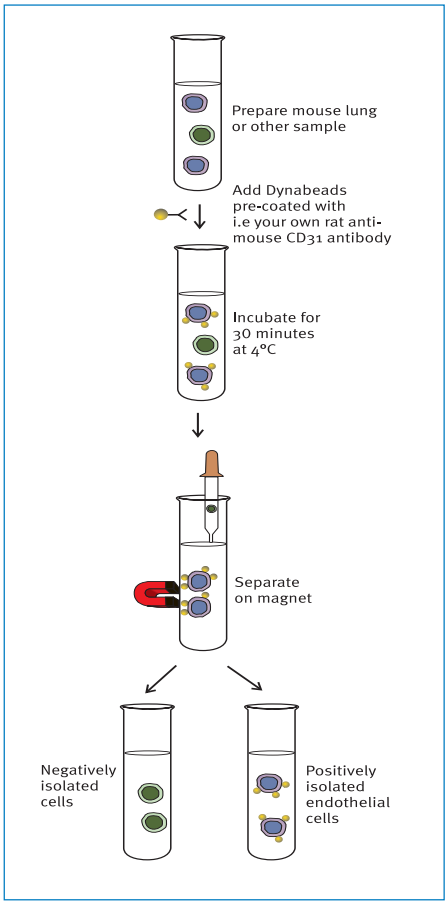

Fig.1. Simple methodology of isolation of pure EC from mouse tissue.

Applications

1. A strategy for endothelial cell isolation from murine tissues

A rapid, reproducible method to isolate murine tissue EC has been developed by Dong *et al* (1). Briefly, remove tissues aseptically, mince into 1 x 2 mm squares and digest with collagenase A. Direct isolation: covalently couple a monoclonal rat anti-mouse CD31 antibody to pre-washed Dynabeads® Sheep anti-Rat IgG overnight. Add the anti-CD31 coated beads to the cell suspension, incubate and mix, then collect bead-bound cells using a magnet (fig.1). Wash bead-bound cells to obtain a high purity and add trypsin/EDTA to release the beads from the cells. Centrifuge the released cells and resuspend in growth medium for cell culture and further cloning. Of 300 cells plated, 29 clones were obtained after two weeks and all clones were positive for CD31. To evaluate the specificity and recovery efficiency of this method, the $CD31^+$ murine EC line H5V was mixed with a melanoma cell line and or mixed with L929 fibroblasts. In both cases the percentage of isolated EC was ›98% and the recovery was 65% of the original $CD31^+$ murine EC.

2. Isolation of primary mouse lung endothelial cells from cultures

Hartwell *et al* (2) developed this method; wash lung tissues, mince, digest with collagenase A and filter through a nylon mesh before centrifuging and plating in F12 (HAM) medium. After 48 hrs, wash the plates and add fresh culture medium. Coat Dynabeads® Sheep anti-Rat IgG with rat anti-mouse intercellular adhesion molecule-2 IgG (PharMingen) and add to the plated cells. After 1 hr of incubation, wash cells with PBS and trypsinize to release all cells. Collect the bead-bound cells with a magnet. Stain EC for P-selectin, an adhesion receptor for leucocytes.

3. Method to establish pure fibroblast and endothelial cell colony cultures from murine bone marrow

Adherent stromal cells of bone marrow consist of EC, fibroblasts and macrophages. EC and macrophages are phagocytic but fibroblasts are not. These differences are used to isolate the different cell populations. Fei *et al* used magnetic separation to obtain cultures of EC and fibroblasts (3). Bone marrow cells were cultured and non-adherent cells poured off. The adherent cells were used in two ways:

To obtain EC, release the adherent cells from the culture plates with trypsin and add Dynabeads® M-450 Epoxy in the ratio of 3 beads: 1 cell and incubate for 2 hrs at 37°C. At this temperature, phagocytic cells in culture engulf the beads. Collect bead-bound cells with the magnet and discard non-phagocytic fibroblasts. Culture bead-bound cells in low concentration for 10 days to reduce macrophage growth. Reculture EC clones (>20 cells) to confluent growth.
To achieve pure fibroblasts use beads in higher quantities. Add a 40 bead: 1 cell ratio to trypsinized adherent cells to ensure all phagocytic cells (EC and macrophages) engulf beads. Collect bead-bound cells with the magnet and remove and culture the non-phagocytic fibroblasts. Re-passage the fibroblasts to ensure there were no contaminating cells and then grow to confluence.

References

1. Q. G. Dong, *et al.*, A general strategy for isolation of endothelial cells from murine tissues -Characterization of two endothelial cell lines from the murine lung and subcutaneous sponge implants. Arterioscler.Thromb.Vasc.Biol. 1997; 17:1599-1604.
2. D. W. Hartwell, *et al.*, Role of P-selectin cytoplasmic domain in granular targeting in vivo and in early inflammatory responses. Journal of Cell Biology 1998; 143 4:1129-1141.
3. R-G. Fei, *et al.*, A method to establish pure fibroblast and endothelial cell colony cultures from murine bone marrow. Exp.Hematol. 1990, 18:953-957.

Materials and Methods

Digestion of lung tissue:

Wash lung tissue in DME, mince into 1-2 mm pieces and digest by adding 20 ml of 0.1% collagenase A at 37°C for 1 hr. Filter the cells through a sterile 40 µm nylon mesh, centrifuge at 100 g for 10 min and plate in F12 (HAM) medium supplemented with 20% fetal bovine serum (FBS), 0.2 U/ml heparin and 5 µg/ml endothelial mitogens (Biomedical Technologies). After 48 hrs, wash the plated cells in PBS and resuspend in fresh culture medium (application 2). In application 1, resuspend the tissue digest in 4 ml 10% FCS-DMEM and use directly for EC isolation.

Coating of Dynabeads® Sheep anti-Rat IgG with rat anti-mouse intercellular adhesion molecule-2 IgG or anti-CD31:

Coat Dynabeads® Sheep anti-Rat IgG by mixing with a rat IgG monoclonal. Pre-wash the beads in PBS and add the antibody (maximum 1.5 µg mAb per 1 x 10^7 beads, 25 ml of beads as supplied). Incubate beads and antibody with mixing at room temperature for 30 min or at 2-8°C overnight. Wash coated Dynabeads® 3 times in PBS/2% FCS, aided by the magnet, to remove excess antibody.

Isolation of EC (application 1):

Add 30 µl of pre-coated Dynabeads® to 1 ml of cell suspension and incubate for 30 min at 2-8°C with mixing. Collect the bead-bound cells using a magnet and wash 5 times in 10% FCS-DMEM and once with FCS-free DMEM and digest for 5-10 min at 37°C in 1 ml of trypsin/EDTA, to release the beads from the cells. Centrifuge the bead-free cells in 10% FCS-DMEM and then resuspend in 7 ml growth medium for culture.

Isolation of cultured EC (application 2):

Add coated Dynabeads® to the cells and incubate for 1 hr at 37°C to allow binding to the cells. Wash the cells once in PBS, trypsinize to release from the plate and collect the bead-bound cells using a magnet. To optimise the purity, wash the bead-bound cells twice using PBS and the magnet.

Note: Use approx. 5 beads per target EC cell (resuspend EC to 2mill/ml and use at least 1 x 10^7 Dynabeads® (25 µl) per ml of sample.

Ordering Information

Product	Kit Components	Prod. No.
Dynabeads® M-450 Epoxy	5 ml	140.11
Dynabeads® Sheep anti-Rat IgG	5 ml	110.35
Dynal MPC®-S	Holds 6 microcentrifuge tubes	120.20
Dynal MPC®-L	Holds 6 5ml FACS tubes or standard blood tubes	120.21

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