Isolation of Multipotent Stromal Cells from Mouse Bone Marrow

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[Abstract] Generating mouse multipotent stromal cells (MSC) from bone-marrow cells is usefull for a wide range of applications. Effectively, these MSC can differentiate into adipocytes, osteocytes [See "Binding to Secreted Bone Matrix *in vitro*" (Tormo *et al.*, 2004)] or chondrocytes upon culture in specific differentiation medium.

Materials and Reagents

1. 6-8 weeks old mouse
2. PBS without Ca^{2+} and Mg^{2+} (Wisent, catalog number: 311-01-CL)
3. Dulbecco's Modified Eagle's Medium High glucose with stable L-glutamine (DMEM) (Wisent, catalog number: 319-015-CL)
4. Fetal bovine serum (FBS) (Life Technologies, Gibco®, catalog number: 12483)
5. Penicillin/Streptomycin solution (Wisent, catalog number: 450-201-EL)
6. Trypan blue (Life Technologies, Gibco®, catalog number: 15250-061)
7. Trypsin 0.05%/EDTA 0.53 mM (Wisent, catalog number: 325-042-EL)
8. APC-conjugated anti-CD31 antibody (clone MEC13.3) (BD biosciences, catalog number: 551262)
9. FITC-conjugated anti-CD45 antibody (clone 30-F11) (BD biosciences, catalog number: 553080)
10. APC-conjugated anti-CD44 antibody (clone IM7) (BD biosciences, catalog number: 559250)
11. PE-conjugated anti-CD105 antibody (clone MJ7/18) (BD biosciences, catalog number: 562759)
12. FITC-conjugated anti-CD90 antibody (clone 5E10) (BD biosciences, catalog number: 555595)

Equipment

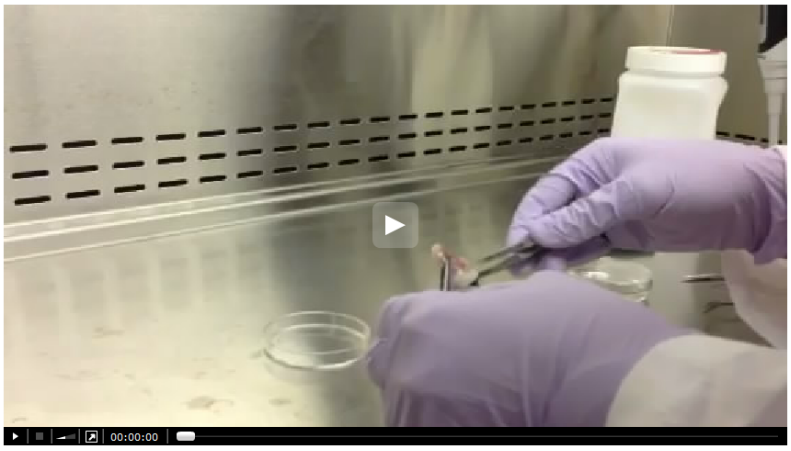
1. Scissors and forceps

2. Syringe 1cc with 27 Gauge x 1-½ needle (BD, catalog numbers: BD-309659 and BD-305109)
3. Petri dishes 100 x 20 mm (BD, catalog number: DL-353003)
4. 50 ml conical tubes (Progene®, catalog number: 71-5000-B)
5. Table top centrifuge
6. Culture hood
7. Hemocytometer
8. T-25 flask (BD, catalog number: 353108)
9. 37 °C, 5% CO_2 Cell culture incubator
10. Flow cytometer (*e.g.* BD LSRFortessa)

Procedure

1. Under culture hood, flush tibia and femora from one 6-8 weeks old mouse with FBS-free DMEM in a dish with DMEM previously warm at 37 °C (see Video 1).

   Video 1. Mouse-bone marrow collection



2. With a syringe and needle, aspirate and eject 2-3 times the bonne marrow to disrupt it.
3. Transfer in a 50 ml conical tube.
4. Centrifuge 10 min at 430 *x g* at 4 °C.
5. Resuspend the pellet with DMEM supplemented with 15% FBS and Penicillin/Streptomycin at a density of 10^6 cells/ml.
6. Plate 1 x 10^7 cells (10 ml) in a T25 flask.
7. Incubate at 37 °C-5 % CO_2 for 7-10 days without changing medium.
8. Remove the supernatant in order to keep only adherent cells and wash the flask 2 times with 5 ml of PBS.

9. Trypsinize cells for expansion. For cells trypsinization: add 0.5 ml of trypsin on the rinsed cells. Wait 3 to 5 min until cells peel off the plastic surface. Transfer cells in a 15 ml tube and add quickly 10 ml of DMEM containing 10% FBS in order to inhibit trypsin action.
10. After 7 - 10 days, assess the phenotype of the growing adherent cells by flow cytometry.
    a. Stain cells with fluorescein isothiocyanate (FITC)-labeled anti-CD45 (clone 30-F11) and with allophycocyanine (APC)-labeled anti-CD31 (clone MEC133). These antibody should be diluted 1/200. Cells should be negative for these two markers as CD45 is a hematopoietic cells marker, and CD31 is an endothelial cells marker.
    b. Stain cells with APC-labeled anti-CD44 (clone IM7), phycoerythrin (PE)-labeled anti-CD105 (clone MJ7/18) and with FITC-labeled anti-CD90 (clone 5E10), all used at a 1/200 dilution. Cells should be positive for these three markers.

Acknowledgments

This protocol is adapted from Tormo *et al*. (2013).

References

1. Tormo, A. J., Beaupre, L. A., Elson, G., Crabe, S. and Gauchat, J. F. (2013). A polyglutamic acid motif confers IL-27 hydroxyapatite and bone-binding properties. *J Immunol* 190(6): 2931-2937.
2. Tormo, A. J., Beauséjour, C. and Gauchat, J. F. (2014). Binding to secreted bone matrix *in vitro*. *Bio-protocol* 4(2): e1030.

