Center for Musculoskeletal Research
Massachusetts General Hospital
50 Blossom Street, Thier 10 & 11
Boston, MA 02114-2696

Email: CSRMAIL@PARTNERS.ORG
Website: www.csr-mgh.org

Musculoskeletal Cell Core
Directors: Paola Divieti Pajevic, MD, PhD
Lou Gerstenfeld, PhD

Email: BoneCell@bu.edu

Bone Marrow Macrophages/Mononuclear Cells Isolation (osteoclast precursors)

Material

- Fycoll Paque plus (**GE cat #71-7167-00 AG**)
- 10 cm Dishes
- 6-8 weeks old mouse
- 70% ethanol
- RANKL (R&D or Shenandoah. Prepare stock at 0.1 mg/ml)
- Mouse M-CSF (R&D or Shenandoah. Prepare stock at 0.1 mg/ml)
- Surgical instruments (small scissors n=2; small forceps n=2, scalpel)
- Bead sterilizer
- Alpha MEM +10%FBS +1 %AA (complete medium)
- 5 and 10mL syringes
- 40 uM cell strainer

Procedure:

Day 1:

For isolation of mouse BMMs from WT mice, femurs and tibias of 4–6-week-old mice are aseptically removed and BMSCs are flushed out the marrow cavity with α-MEM (no serum added) or sterile PBS (usually 5 ml for each bone).

The Center for Musculoskeletal Research is supported by NIH NIAMS P30 AR075042

Cells are collected in a 50ml tube, centrifuged at 1000 RPM for 5-10 min and supernatant is removed and pellet is gently resuspended in 10 ml of α-MEM containing 10% FBS 1% AA and cultured in 10 cm dishes at 37 °C and 5% CO2 *for 24-48 hr* .

Day 2:

Gently remove non-adherent cells with a 10 ml pipette and transfer to a new 50 ml conical tube. Wash gently the dish one more time with 10ml of α-MEM containing 10% FBS 1% AA and add to the same 50-ml tube

Centrifuge at 1000RPM for 10 min

Suspend the cells in 5 ml of complete medium (α-MEM containing 10% FBS 1% AA). Use another 5 ml of medium to rinse the tube.

In a new 50ml conical tube add 15 ml of Fycol hypaque (Farmacia) and gently layer the 10ml of cell suspension

Spin cells at 1500 rpm for 15 min

Pull off the first 10ml of medium off the top and collect the interface with a 10 ml pipette into a 50 ml conical tube

Bring up the volume to 50 ml with complete medium.

Centrifuge for 10 min at 1000RPM and resuspend the cells in α-MEM containing 10% FBS 1% AA. 2 ml/mouse.

Cells are now ready for downstream application.

For osteoclastogenesis:

Count the cells and plate at 20,000 cells/well in 96 well plate or 50,000 cells/well in 12 well plate and treat with MCSF for 48-72 hr (MCSF 20-50 ng/ml)

After 48-72 hr start RANKL treatment (50 ng/ml), in addition to MCSF treatment. Replace medium every 3 days

Osteoclasts usually form after 2-3 days in RANKL

The Center for Musculoskeletal Research is supported by NIH NIAMS P30 AR075042

TC setting

The Center for Musculoskeletal Research is supported by NIH NIAMS P30 AR075042