

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

Ear Mesenchymal Stem Cells Isolation and Culture

Materials

- HBSS (Invitrogen 14025-092) or sterile PBS
- Primocin (Invivogen ant-pm-1)
- 70% EtOH
- Collagenase I (Worthington Biochemical Corp. LS004196)
- RBC Lysing Buffer (Sigma R7757)
- DMEM/F12 (Invitrogen 11330 032)
- FBS
- FGF Basic rec. murine (Peprotech 450-33)

eMSC culture medium

- **85ml** DMEM/F-12
- **15ml** FBS
- **200ul** Primocin
- **100ul** 10ug/ml FGF Basic

Note: The eMSC have been cultured without FGF successfully.

Procedure

- Set centrifuge at 28C and shaking water bath at 37C.
- Prepare 2 x 50ml conical tubes per genotype
 - Tube 1: 10ml **70% EtOH**
 - Tube 2: 10ml **HBSS**
- Prepare **Collagenase I**: 2mg/ml in sterile **HBSS**. Make 30ml.
- Sacrifice mouse (3-wk-old) and collect ears. Try to trim as much as the excess hair as possible.
- Submerge in Tube 1 for about 2 minutes.
- Transfer to Tube 2
- Move the tubes to the Tissue Culture hood.
- Place the ears in a small petri dish containing 500ul of Collagenase I.
- Chop off and mince little pieces of ears using scissors and tweezers.
- Add 500ul of **Collagenase I** to wash the petri dish and pour everything in a 50ml conical tube.

Note: If there is tissue remaining in the dish, add 1ml of Collagenase I, pour in the tube (don't go over 10ml of collagenase I).

- Digest for 1hr at 37C in the shaking water bath. Give the tubes a shake every 10min.
- Filter through 70 to 100um cell strainer.
- Centrifuge at 1600rpm for 10min at 28C.
- Discard supernatant.
- Resuspend pellet in 1ml of **RBC lysis Buffer**.
- Mix vigorously by pipetting up and down and let it sit for 1min.
- Add 10ml of **culture media**.
- Centrifuge at 1600rpm for 10min at 28C.
- Discard supernatant.
- Resuspend the pellet in 2ml of **culture media** (2ml = 2 ears) and seed all the cells in one well of 6-well plate (1well = cells from 2 ears).
- Change culture media every 2-3 days.

Note: it may take up to 2 days for colonies to appear. On Day 3, replace with 2ml of media or Trypsinize and place in 10cm dish if the cells are almost 100% confluent.