

Isolation of murine (C57B6/J) bone marrow derived stromal cells (mMSC)

Adapted from Peister et al. Blood. 2004 Mar 1;103(5):1662-8.

Extract BM from femur and tibia of C57B6/J mice and flush in same manner as for HSC isolation.

Initial Culture conditions

Plate all cells from BM harvest into large T175 flask.

Media requirements:

CIM: RPMI-1640 (Invitrogen) --- inhibits hematopoietic cells in culture
9% FBS (Gibco)
9% HS (Gibco)
1% pen-strep (Gibco)
Trypsin-EDTA to lift cells

Passage 1: 4 weeks w/media exchange every 3-4 days.

Passage 2: 1-2 weeks w/media exchange every 3-4 days. Following passage 2, trypsinize cells and replat at 50cells/cm² in T175 to expand.

MSC: IMDM (Invitrogen) --- selected on media test for C57B1/6J
10% FBS (Gibco)
10% HS (Gibco)
1% pen-strep (Gibco)
Trypsin-EDTA to lift cells

Passage 3: 1-2 weeks (or until enough MSCs for use or freeze) w/media exchange every 3-4 days.

Freezing media:

MSC complete media (above) with final 5%DMSO concentration, freeze in controlled slow-freeze chamber in -70C O/N, then transfer to LN2 for storage indefinitely.

Differentiation assays

Adipogenesis – IMDM, 10% FBS, 10% HS, 100U/mL pen, 100µg/mL strep, 5µg/mL insulin (Sigma), 50µM indomethacin (Sigma), 1e-6M dexamethasone, 0.5µM 3-isobutyl-1-methylxanthine (IBMX-Sigma).

Incubate 3 weeks w/media exchange twice/week. Cells fixed w/10% formalin 20' RT, stained w/0.5% Oil Red O (Sigma) in MeOH (Sigma) 20' RT.

Osteogenesis – IMDM, 10% FBS, 10% HS, 100U/mL pen, 100µg/mL strep, 20mM β-glycerol-phosphate (Sigma), 50ng/mL thyroxine (Sigma), 1nM dexamethasone (Sigma), 0.5µM ascorbate 2-phosphate (Sigma).

Incubate 3 weeks w/media exchange twice/week. Cells fixed w/10% formalin 20', stained w/Alazarin Red pH 4.1 (Sigma) 20' RT.

Immunostaining:

Antibodies from BD: CD11b-FITC, CD34-PE, CD45-FITC, Ter-119-PE, CD45R/B220-FITC, Ly6G-FITC, Ly-6C-FITC, CD3eFITC, FLK1-PE (VEGF2), CD31-FITC (PECAM), CD90-PE (Thy1), CD117-APC (c-kit), CD106-FITC (VCAM-1), Ly-6A/E-FITC (Sca-1)