

# A Simple Robust Method to Highly Enrich Mesenchymal Progenitor Cells from Compact Bone

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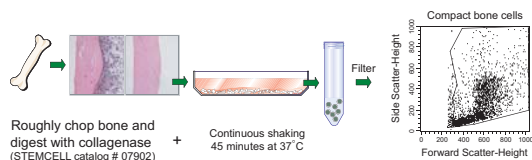
## Introduction

Mesenchymal Progenitor Cells (MPC) have been implicated as potential targets in a range of cellular therapies for the treatment of defects of both the hematopoietic and skeletal systems and as vehicles for gene therapy. In order to utilize the potential of these cells in various therapies, a pre-clinical animal model in which both the biology and potential therapeutic applications of these cells can be assessed is of fundamental importance. The goal of the current study was thus to develop a simple, robust and reproducible method for the isolation of Mesenchymal Progenitors from mouse compact bone (CB).

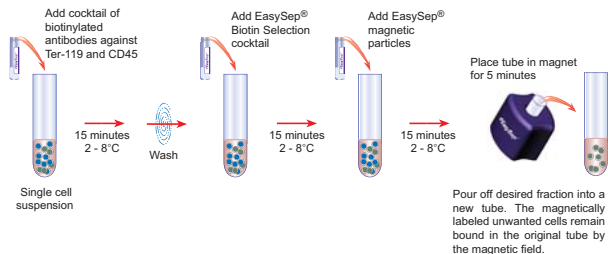
## Methods

CB cells were harvested from tibiae and femurs of C57BL6/J mice.

**Figure 1.** Isolation of MPC from mouse CB (Technical Manual available, STEMCELL catalog # 28374)



**Figure 2.** EasySep<sup>®</sup> procedure for column-free enrichment of MPC (STEMCELL catalog # 19771)



## Cell Culture

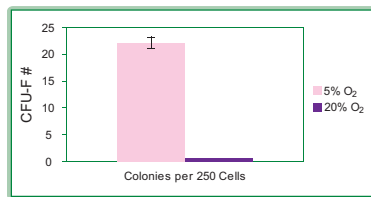
**Colony forming unit-fibroblast (CFU-F) assay:** Pre-enriched CB cells were plated in a 6-well plate at densities ranging from 10,000 - 100,000 cells/well. Enriched CB cells were plated at 250 - 2,500 cells/well and cultured for 10 - 13 days.

**Expansion assay:** CB-enriched cells were plated in a 6-well plate at densities ranging from 10,000 - 50,000 cells/well. For both CFU-F and expansion assay, cells were cultured in Complete MesenCult<sup>®</sup> medium (mouse) with 5% Oxygen (in Hypoxia chamber; STEMCELL catalog # 27310). Passage 2 (P2) CB-enriched cells were differentiated into adipocyte, chondrocyte or osteogenic cells and stained with Oil red O stain, Alcian Blue or alkaline phosphatase, respectively.

**Long-term culture initiating cell (LTC-IC) assay** was used to examine the ability of CB-enriched cells to support maintenance of primitive hematopoietic cells. Enriched hematopoietic mouse cells from bone marrow (SCA-1+ cells; 92% purity) isolated with EasySep<sup>®</sup> Mouse SCA-1 Positive Selection Kit were plated under limiting dilution on irradiated feeders (P2 CB-MPC plated at 10,000 cells/well or primary bone marrow cells plated at 3 x 10<sup>5</sup> cells/well). Cells were cultured in MyeloCult<sup>™</sup> mouse long term culture medium supplemented with 10<sup>-6</sup> M hydrocortisone (LTCM) medium at 33°C and 5% CO<sub>2</sub> for 10-14 days. LTC-IC frequency was calculated using L-Calc<sup>™</sup> after 4 weeks.

## Results

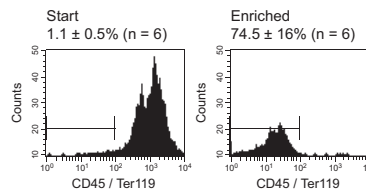
**Figure 3.** Culture in low (5%) oxygen leads to a 35-fold increase in CFU-F derived colony numbers from enriched mouse MPC CB



CFU-F numbers from CD45 / Ter-119 depleted CB fraction; n=3. Cultures maintained in a Hypoxia Chamber (STEMCELL catalog # 27310) gassed with 5% O<sub>2</sub> / 10% CO<sub>2</sub> / 85% N<sub>2</sub>

CB is a richer source of MPC than bone marrow. When cultured under 5% O<sub>2</sub>, frequency of CFU-F colony number is higher in CB (433 ± 225 / 10<sup>6</sup> cells) than in marrow (11.7 ± 3.5 / 10<sup>6</sup> cells); n = 3.

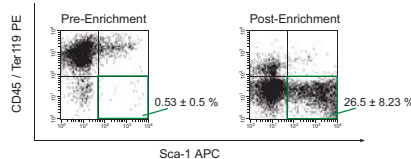
**Figure 4.** Enrichment of MPC by immunomagnetic depletion of CD45<sup>+</sup> and Ter-119<sup>+</sup> cells



**Table 1.** CFU-F Enrichment and Recovery

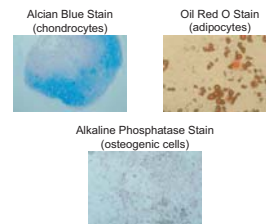
| n | Fold Enrichment | % Recovery |
|---|-----------------|------------|
| 6 | 110 ± 74        | 58 ± 19    |

**Figure 5.** Sca-1 expression on CB cells pre- and post-enrichment

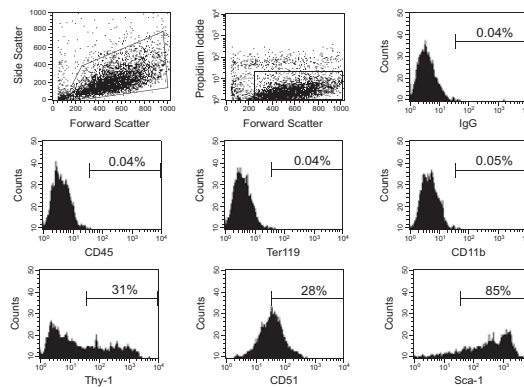


Fold enrichment of Sca-1+ cells = 50 fold.

**Figure 6.** Differentiation of CB-enriched cells into adipocytes, chondrocytes, and osteogenic cells



**Figure 7.** Phenotype of enriched mouse MPC from CB culture at Passage 2 (P2)



Cultured MPC from mouse CB (P2) express Thy-1 (31% positive cells), CD51 (28% positive cells) and Sca-1 (85% positive cells) but do not express hematopoietic markers (<0.1%).

**Table 2.** LTC-IC frequency (preliminary results)

| LTC-IC Frequency (Range) in mouse BM SCA-1 <sup>+</sup> cells |                         |
|---|-------------------------|
| CB-MPC Feeders  | 1 in 2200 (1900 - 2500) |
| Primary BM Feeders  | 1 in 1200 (900 - 1500)  |

## Conclusions

- Culture of MPC in low oxygen is crucial for optimal CFU-F formation
- MPC can be highly enriched from CB using a simple and rapid (<1 hr) EasySep<sup>®</sup> magnetic separation procedure
- CB enriched MPC are capable of supporting primitive hematopoietic cells in long-term culture



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