Supplementary File

Characterization of rat bone marrow stromal cell

Method: Flow cytometry was performed at the Bioengineering Flow Cytometry Core Facility at the University of Maryland. Both harvested bone marrow and 3rd passaged cells were then placed in red cell lysis buffer (10 mM $KHCO_3$, 150 mM NH_4Cl, 0.1 mM EDTA) for 10 min and washed once with complete media. Approximately 1×10^6 cells were transferred to a 5 ml BD Falcon polystyrene tube and labeled for the following antibodies: CD45-Alexa Fluor 647 (clone OX-1, Biolegend, San Diego, CA); CD29-FITC (clone Ha2/5, BD Biosciences, San Jose, CA); CD90-PE (clone OX7, Santa Cruz Biotech, Santa Cruz, CA); CD34-PE-Cy7 (clone ICO115, Santa Cruz Biotech); and CD44 (clone OX49, Santa Cruz Biotech) with goat anti-mouse Alexa Fluor 700 (Invitrogen, Carlsbad, CA), as well as unstained and appropriate isotype controls, and a panel stain including all surface markers. Each tube was washed with 2 mL FACS staining buffer (1x PBS/1% FBS) and centrifuged for 5 min at 500 rpm at 4°C. The supernatant was discarded and the pellet was resuspended in 200 µl FACS staining buffer and the appropriate antibodies were added. The tubes were incubated on ice for 20 min during antibody binding in the dark and then washed twice with 2 ml of PBS and centrifuged for 10 min at 500 rpm at 4°C. The supernatant was discarded and the pellet was resuspended in the final volume of 500 µl. The samples were analyzed on a BD FACSAria II flow cytometer BD Biosciences (San Jose, CA) and the data was analyzed using the BD FACSDiva software.

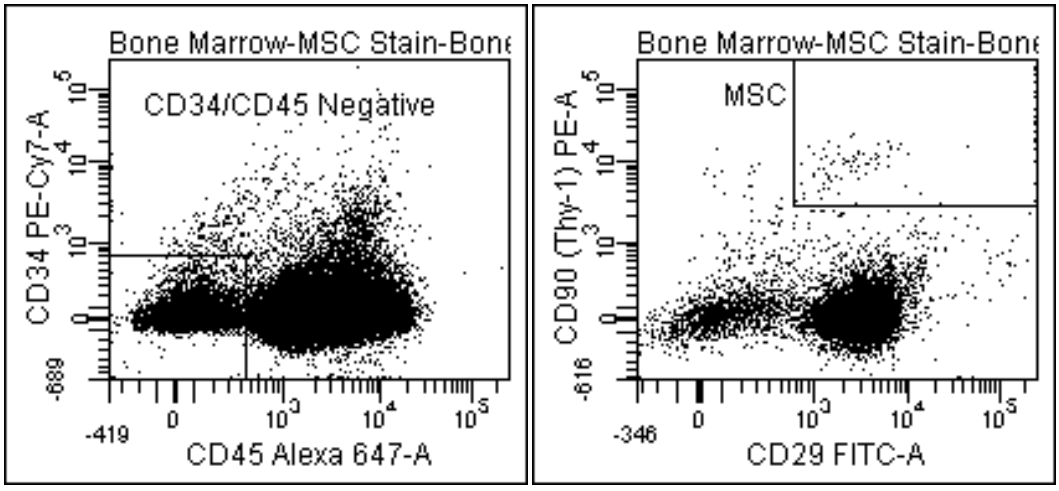
Result: The mesenchymal stem cell population in a heterogeneous bone marrow stromal cell sample can be characterized by cell surface marker staining. In particular, MSCs are thought to be positive for CD29 and CD90, while negative for CD34 and CD45 [50, 51]. Results showed that freshly harvested BMSCs are a heterogeneous cell population including leukocytes, monocytes, and granulocytes. Bone marrow aspirate contained 86.7% of CD45(+) cells, but this percentage fell to 17.0% after three passages (Supplementary table). The percentage of CD29(+) and CD90(+) cells increased after 3 passages (92.1 to 100 % and 76.4 to 80.1 %, respectively). The percentage of the total mesenchymal stem cell population, defined here as CD29(+), CD90(+), CD34(-), and CD45(-), was only 0.1% for freshly harvested BMSCs, however it was 49.6% for subcultured cells. As shown in supplementary figure, it is concluded that a subculture procedure is suitable for expanding rat MSC populations from primary bone marrow stromal cells.

Supplementary Table: FACS characterization of the mesenchymal stem cell population (as defined by CD29(+)/CD90(+)/CD34(-)/CD45(-)) of fresh bone marrow stromal cells and those cells after three passages.

	Cell Population Composition	
Cell Surface Markers	**Fresh Bone Marrow Cells**	**Passage 3 Cells**
CD34	0.4%	1.1%
CD45	86.7%	17.0%
CD29	92.1%	100.0%
CD90	76.4%	80.1%
CD34-/CD45-/CD29+/CD90+	0.1%	49.6%

Supplementary Figure: Flow cytometry analysis of freshly derived BMSCs from rats (A) and subcultured cells after three passages (B). Approximately 50% of the BMSCs presented MSC markers (CD29(+)/CD90(+)/CD34(-)/CD45(-)) after three passages.

(A)



(B)