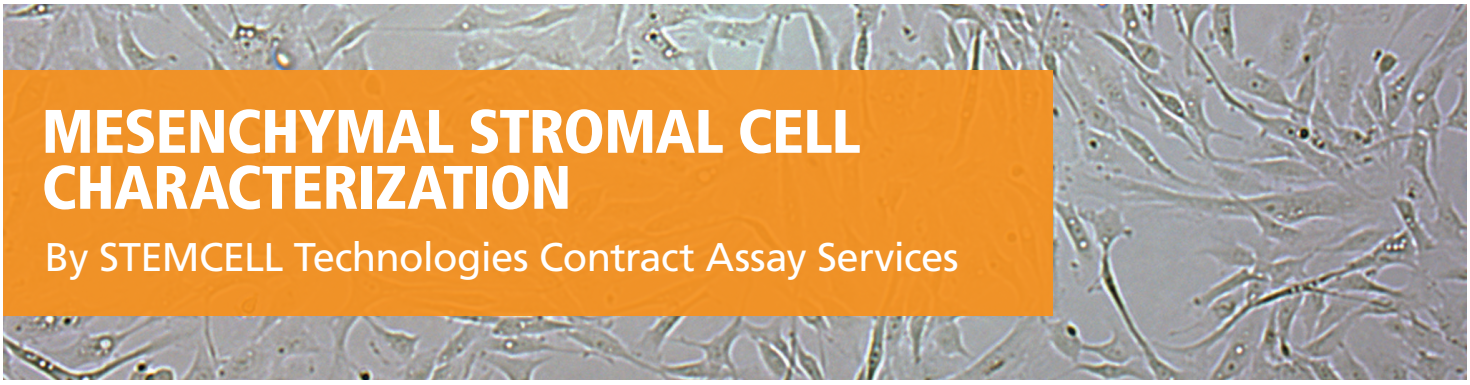


MESENCHYMAL STROMAL CELL CHARACTERIZATION

By STEMCELL Technologies Contract Assay Services

Characteristics of MSCs

Human multipotent mesenchymal stromal cells (also termed mesenchymal stem cells, MSCs) are a heterogeneous population of plastic-adherent, fibroblast-like cells, which can self-renew and differentiate into bone, adipose, and cartilage tissue in culture. These cells can be isolated from different tissues including bone marrow (BM), adipose tissue, umbilical cord matrix, tendon, lung, and dental pulp.[1]

The International Society for Cellular Therapy (ISCT) has defined four minimal criteria for MSCs:

i. Adherence to plastic under standard tissue culture conditions.

ii. Expression of CD105, CD73, and CD90.

iii. Lack of expression of CD45, CD34, CD14/CD11b, CD79/CD19, and HLA-DR surface markers.

iv. Differentiation into adipocytes, osteoblasts, and chondroblasts in vitro.[2]

Nevertheless, the MSC phenotype can be variable due to the variety of potential tissue sources and the differences in cell isolation and culture procedures used. This phenotypic variability underscores the importance of characterizing MSCs to accurately assess their ability to expand and differentiate, as well as their immunomodulatory capacity, particularly in the light of increased interest in their therapeutic applications.

Therapeutic Potential of MSCs

Expanded multipotent MSCs are being extensively studied for their possible therapeutic properties in numerous preclinical and clinical settings. Researchers initially focused on using the stem cell-like properties of MSCs for tissue regeneration and repair. However, it is now well-established that their beneficial effects are mostly derived from their ability to modulate the inflammatory response.

Systemic infusion of MSCs has proved beneficial in different preclinical models of acute lung injury, myocardial infarction, diabetes, and renal and hepatic failure. Some of the human conditions for which the safety and efficacy of MSC-based therapies are being, or will soon be, studied in clinical trials include acute graft-versus-host disease, multiple sclerosis, osteogenesis imperfecta, stroke, spinal injury, systemic lupus erythematosus, and cardiovascular disease.

Customized MSC Characterization by STEMCELL's Contract Assay Services

- Characterization of phenotype by flow cytometry.
- Assessment of growth or doubling time.
- Quantification of differentiation potential into adipocytes, osteoblasts, and chondrocytes.
- Evaluation of immunosuppressive function.

MSC Phenotypic Characterization

MSCs can be characterized by flow cytometry using fluorochrome-conjugated antibodies against cell surface markers. Our customizable flow cytometry panel can help assess the purity of MSCs on freshly thawed cell samples.

Table 1. Negative and Positive BM-Derived MSC Flow Cytometry Marker Panel

Standard Panel	
Negative HSC Marker	**Used to Exclude**
CD34	Primitive hematopoietic cells and endothelial cells
CD45	Leukocytes
CD11b	Monocytes and macrophages
CD19	B cells
HLA-DR	Antigen-presenting cells and lymphocytes
Positive MSC Marker	**Biological Role**
CD73/5'-nucleotidase	Catalyzes production of extracellular adenosine from AMP
CD90/Thy1	Wound repair, cell-cell matrix interactions
CD105/endoglin	Vascular homeostasis; modulates TGFβ functions via interaction with TGFβ RI and TGFβ RII

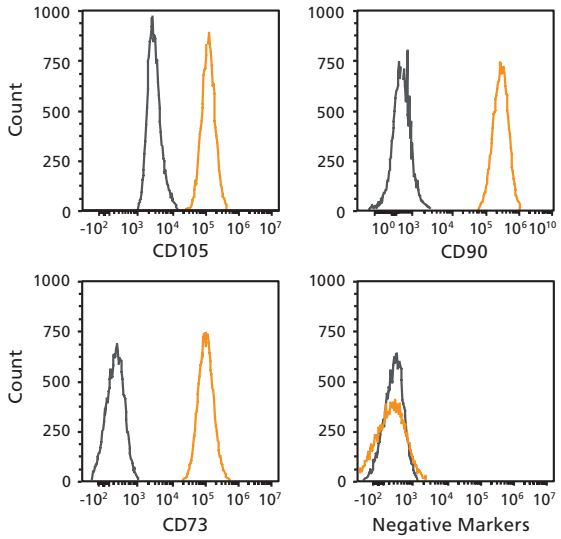


Figure 1. Flow Cytometry Analysis of MSCs

Human BM-derived MSCs were stained with antibodies against positive and negative MSC markers (orange lines) or isotype controls (gray lines). Dead (7-AAD$^+$) cells were excluded and cells were gated based on the light scatter characteristics of MSCs.

MSC Growth Characterization

MSC samples can be quantitatively assessed for their proliferation and doubling rates using the proliferation assay. MSCs are expanded for 6 - 8 passages in MesenCult™ medium. The number of MSCs are evaluated during each passage and the doubling rate is calculated.

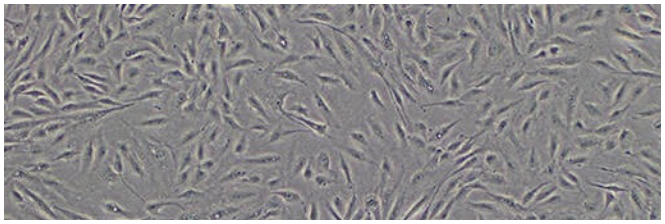


Figure 2. BM-Derived MSCs Cultured in MesenCult™-ACF Medium

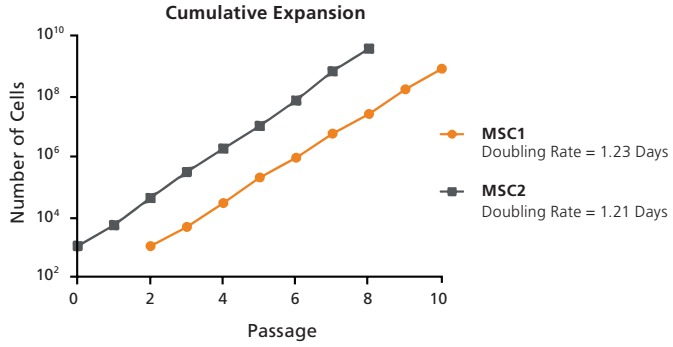
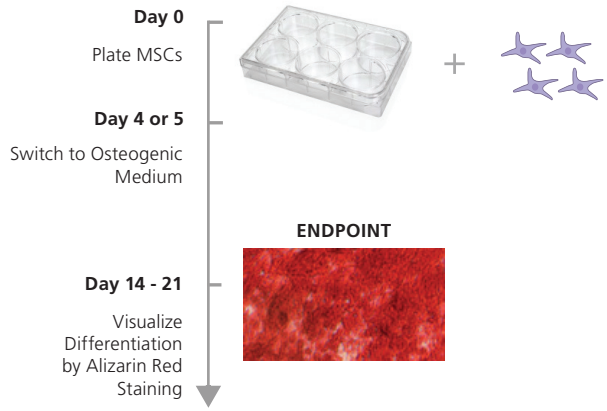


Figure 3. Cumulative Cell Expansion of BM-Derived MSCs in MesenCult™-ACF over 8 passages

Doubling Rate = N / ($\log^2$ (C2 / C1)), where N = total number of days in culture, C1 = initial cell concentration, and C2 = final cell concentration.

Osteogenic Differentiation Assay

MSC samples can be cultured and differentiated into the osteogenic lineage using the MesenCult™ Osteogenic Differentiation Kit (Human) (Catalog #05465). The differentiation is visualized by Alizarin Red staining which detects calcium deposits during mineralization of the osteogenic cells. The dye is extracted from the cultures to quantify the level of osteogenic differentiation.



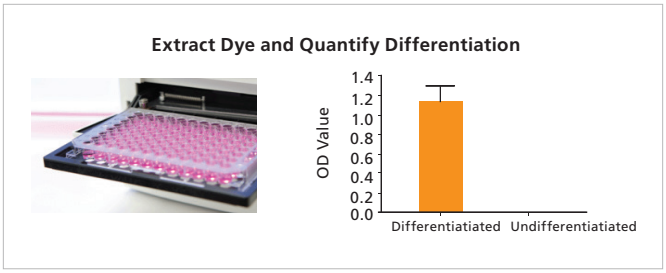


Figure 4. MSC Osteogenic Differentiation Workflow

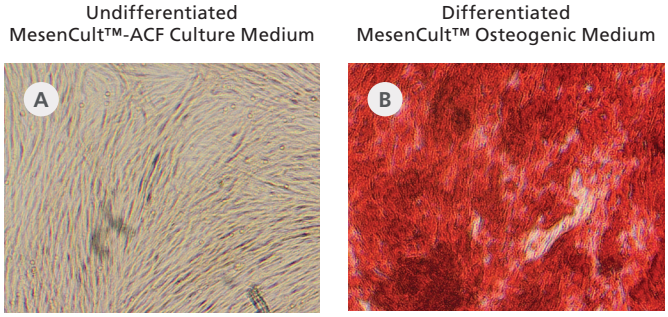


Figure 5. Robust BM MSC Osteogenic Differentiation is Achieved in 14 - 21 days

Osteogenic differentiation was observed within 14 days of induction as indicated by strong calcium (Alizarin Red S) staining. Negative controls of undifferentiated MSC cultures were maintained in MesenCult™-ACF culture medium for the same time period and showed no calcium deposits.

Adipogenic Differentiation Assay

MSC samples can be cultured and differentiated into the adipogenic lineage using MesenCult™ Adipogenic Differentiation Medium (Human) (Catalog #05412). The differentiation is visualized by Oil Red O staining which detects the presence of lipids in adipogenic cells. The dye is extracted from the cultures to quantify the level of adipogenic differentiation.

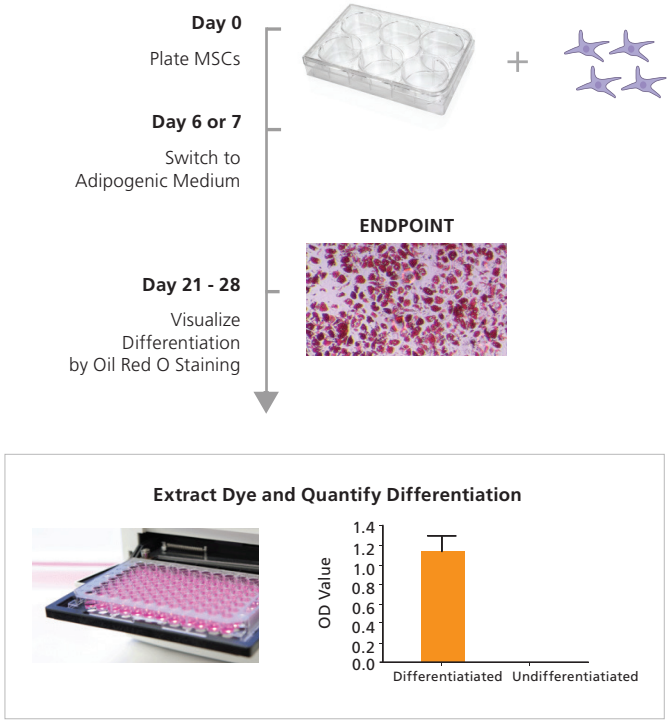


Figure 6. MSC Adipogenic Differentiation Workflow

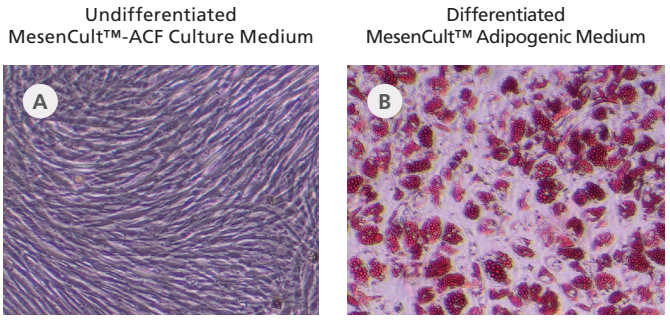


Figure 7. Robust BM MSC Adipogenic Differentiation is Achieved in 21 - 28 days

Adipogenic differentiation was observed within 14 days of induction as indicated by the strong presence of lipids (Oil Red O staining). Negative controls of undifferentiated MSC cultures were maintained in MesenCult™-ACF culture medium for the same time period and showed no presence of lipid.

Chondrogenic Differentiation Assay

MSC samples can be cultured and differentiated into the chondrogenic lineage using MesenCult™-ACF Chondrogenic Differentiation Medium (Catalog #05455). The differentiation and the formation of bone cartilage is visualized by Alcian Blue which detects aggrecan within the cartilage matrix.

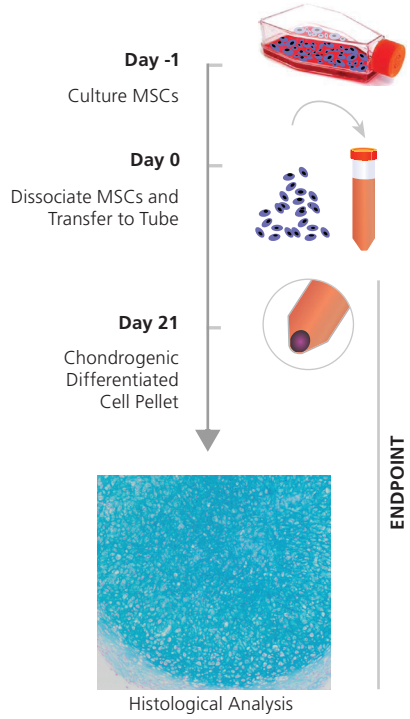


Figure 8. MSC Chondrogenic Differentiation Workflow

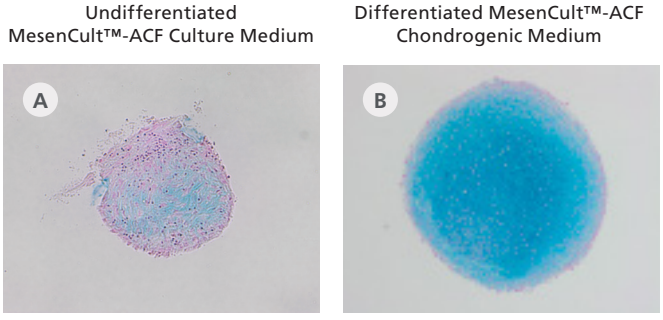


Figure 9. Histology of BM MSC-Derived Chondrogenic Cell Pellet, Day 21

Cell pellet samples were fixed, paraffin embedded, sectioned, and stained with Alcian Blue, which detects aggrecan within the cartilage matrix. Nuclear Fast Red was used as the nuclear counterstain.

MSC T Cell Suppression Assay

MSCs are endowed with remarkable immunoregulatory properties, which make them ideal candidates for cellular therapies. When co-cultured in vitro, they modulate the responses of various immune cell types. The immunosuppressive function of an MSC sample can be evaluated in the suppression assay where CD4+ T cells are stimulated to proliferate in the presence of MSCs, followed by flow cytometry after 4 - 5 days of stimulation.

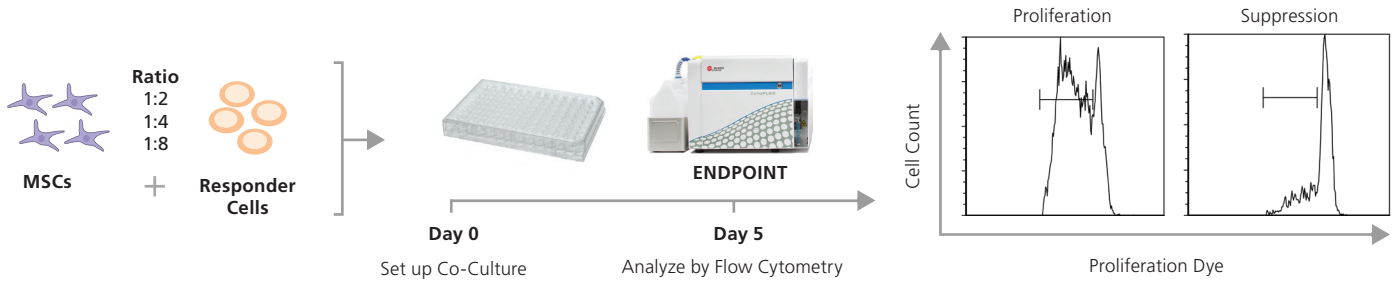


Figure 10. MSC Suppression Assay Workflow

Human BM-derived MSCs were incubated with proliferation dye-labelled responder cells at three different ratios (1:2, 1:4, and 1:8). ImmunoCult™ Human CD3/CD28 T Cell Activator (Catalog #10971) was added to the culture for 4-5 days. After the stimulation, the responder cell proliferation was assessed by flow cytometry.

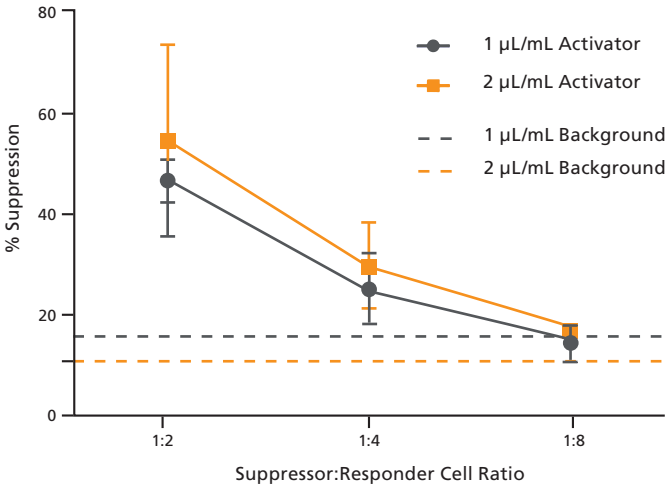


Figure 11. MSC Suppression of CD4+ T Cell Proliferation

Human BM-derived MSCs were able to suppress CD4+ T cell proliferation in a dose-dependent manner. As a background control, the responder cells were cultured at two times the cell concentration to determine baseline suppression in the presence of same amount of total cells as the 1:2 MSC:Responder cell cultures.
% Suppression = 100 – ((% proliferation of responder cells alone – % proliferation of responder cells treated with MSCs) / % proliferation of responder cells alone) x 100.

Sample Requirements

Human biological samples received by STEMCELL must be collected according to the client's institutional review board (IRB) guidelines and local and federal requirements. Such samples must be from donors screened negative for infectious diseases, including but not limited to HIV, HBV, and HCV. Testing documentation must be provided to STEMCELL upon request. For human biological material that has undergone further processing, documentation confirming its mycoplasma-free status must be provided.

Contact us at **contractassay@stemcell.com** for custom assays and clinical trial support.

References

1. Crisan M et al. (2008) Cell Stem Cell 3(3): 301–13.
2. Dominici M et al. (2006) Cytotherapy 8(4): 315–7.