Characterization of Human Bone Marrow- and Adipose Tissue-Derived Mesenchymal Stromal Cells in an Improved Animal Component-Free Culture Medium

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INTRODUCTION

We characterized mesenchymal stromal cells (MSCs) derived from human bone marrow (BM), adipose (AD) tissue, and umbilical cord (UC) tissue by culturing in MesenCult™-ACF Plus Medium (MACF-P), an improved animal component-free (ACF) culture medium. The clonogenic growth of BM mononuclear cells (BM-MNCs) or AD-derived stromal cells (AD-SCs) cultured in MACF-P or control medium containing fetal bovine serum (FBS) was determined by plating at low density in the colony-forming unit-fibroblast (CFU-F) assay. The proliferative potential of BM-, AD-, and UC-derived MSCs was measured after long-term culture in MACF-P or in serum containing medium. To measure the differentiation potential of MSCs, cells were cultured under conditions that stimulate their differentiation into adipogenic, osteogenic, and chondrogenic cells. Finally, the phenotype of BM-, AD-, and UC-derived MSCs cultured in MACF-P was assessed by flow cytometry, and the immunosuppressive function of MSCs was evaluated by a CD4+ T cell proliferation assay in a co-culture system.

Our data demonstrate that MACF-P medium supports optimal derivation of BM- and AD-derived MSCs under completely ACF conditions. The long-term expansion of BM- and AD-derived MSCs was significantly higher in MACF-P than in FBS-containing medium. MSCs differentiated efficiently in vitro under appropriate conditions into adipogenic, osteogenic, and chondrogenic cells, and BM-derived MSCs cultured in MACF-P exhibited immunosuppressive activity in vitro.

MATERIALS AND METHODS

CFU-F Assay

MSCs derived from primary BM-MNCs or AD-SCs were plated at 1 - 5 x 10^4 cells/cm^2 or 0.5 - 4 x 10^3 cells/cm^2, respectively and cultured in MACF-P or FBS-containing medium. After 10 - 14 days, colonies of adherent cells generated by clonogenic progenitor cells were counted.

Cell Expansion

BM-, AD-, and UC-derived MSCs were plated at 1.5 - 3 x 10^3 cells/cm^2 in MACF-P or FBS-containing medium for long-term cell expansion. The proliferative potential of MSCs was measured by counting cells at each passage (P) up to P8 (BM- and UC-derived MSCs) or P9 (AD-derived MSCs).

Differentiation Assays

BM- and AD-derived MSCs expanded in either MACF-P or FBS-containing medium were differentiated into adipogenic, osteogenic, or chondrogenic cells using standard differentiation protocols and visualized by Oil Red O, Alizarin red, and Toluidine blue staining, respectively.

T Cell Proliferation Assay

The in vitro immunosuppressive activity of BM-derived MSCs was investigated in a CD4+ T cell proliferation assay by co-culturing with peripheral blood mononuclear cells (PBMCs). BM-derived MSCs were cultured in MACF-P for two passages, and after harvesting were treated with 25 µg/mL mitomycin C at 37°C for 30 minutes. Subsequently, PBMCs were isolated from a leukapheresis sample and labeled with eFluor™ 450. BM-derived MSCs were then co-cultured with labeled PBMCs at ratios of 1:2, 1:4, and 1:8 MSCs:PBMCs. T cell proliferation in the co-culture was induced by addition of ImmunoCult™ Human CD3/CD28 T Cell Activator at concentrations of 0, 2, and 4 µL/mL on day 0. On day 5, the cells in the co-culture were stained with an anti-CD4-APC antibody, and CD4+ T cell proliferation was evaluated using flow cytometry.

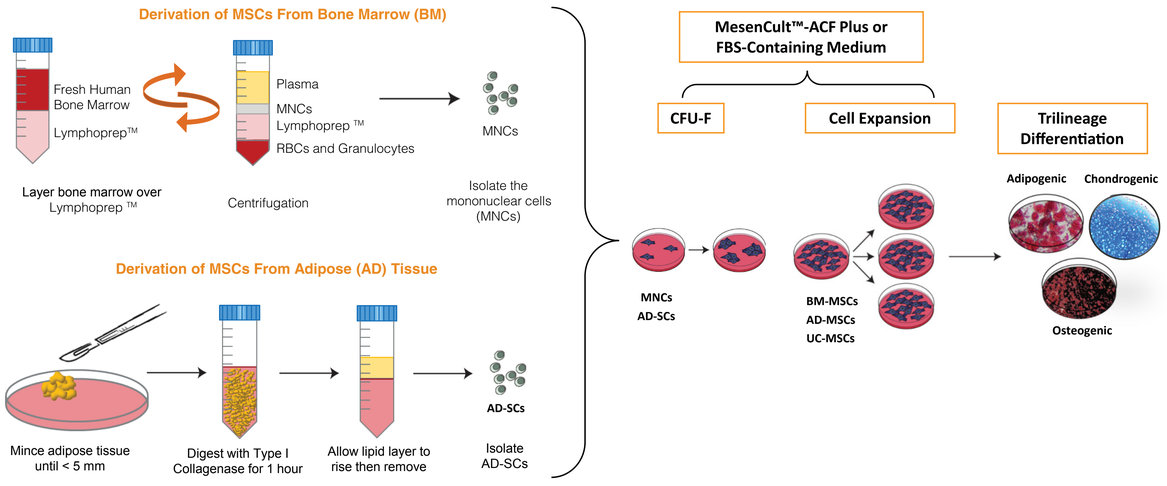


FIGURE 1. Derivation, Expansion, and Differentiation of Tissue-Derived MSCs Cultured in MesenCult™-ACF Plus or in FBS-Containing Medium

RESULTS

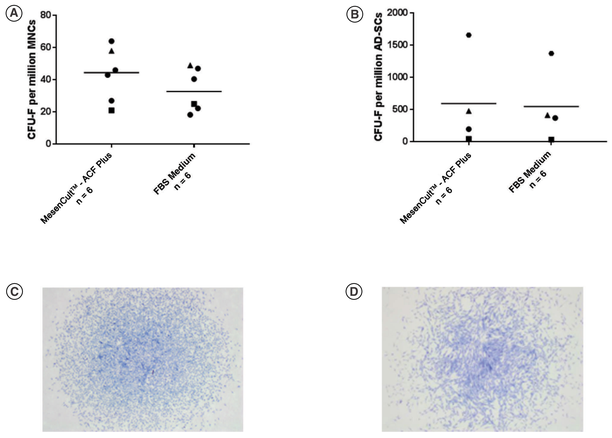
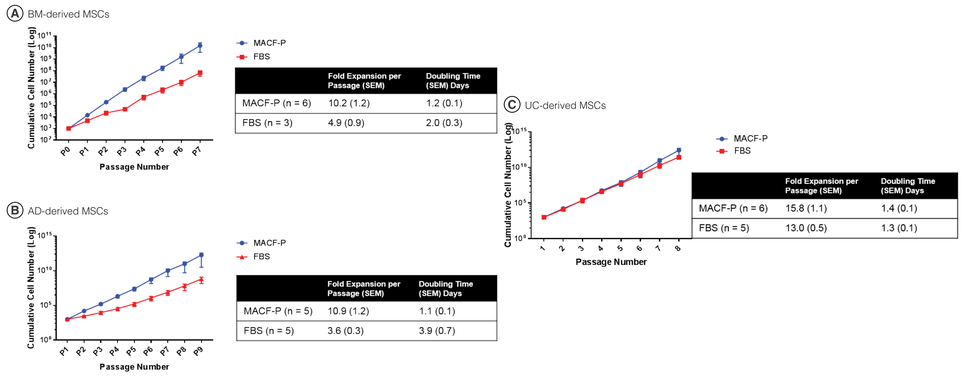


FIGURE 2. MesenCult™-ACF Plus Supports the Derivation of MSC Progenitors from BM-MNCs and AD-SCs Without the Use of Human Serum
(A) The average number of CFU-F per million BM-MNCs was comparable in MesenCult™-ACF Plus and FBS-containing medium.
(B) The average number of CFU-F per million AD-SCs was also comparable in MesenCult™-ACF Plus and in FBS-containing medium.
(C) Representative image of a BM-derived CFU-F colony in MesenCult™-ACF Plus and
(D) in FBS-containing medium.



	Fold Expansion per Passage (SEM)	Doubling Time (SEM) Days
MACF-P (n = 6)	10.2 (1.2)	1.2 (0.1)
FBS (n = 3)	4.9 (0.9)	2.0 (0.3)

	Fold Expansion per Passage (SEM)	Doubling Time (SEM) Days
MACF-P (n = 5)	10.9 (1.2)	1.1 (0.1)
FBS (n = 5)	3.6 (0.3)	3.9 (0.7)

	Fold Expansion per Passage (SEM)	Doubling Time (SEM) Days
MACF-P (n = 6)	15.8 (1.1)	1.4 (0.1)
FBS (n = 5)	13.0 (0.5)	1.3 (0.1)

FIGURE 3. MesenCult™-ACF Plus Supports Superior Expansion of MSCs
Expansion of **(A)** BM-derived MSCs and **(B)** AD-derived MSCs compared to FBS-containing medium. **(C)** The expansion of UC-derived MSCs in MesenCult™-ACF Plus (MACF-P) is equivalent to FBS-containing medium (FBS).

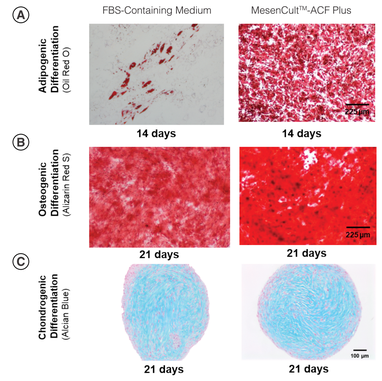


FIGURE 4. Trilineage Differentiation Potential of AD-Derived MSCs Cultured in MesenCult™-ACF Plus or FBS-Containing Medium
AD-derived MSCs can differentiate into **(A)** adipogenic, **(B)** osteogenic, or **(C)** chondrogenic cells using MesenCult™ Adipogenic Differentiation Kit, MesenCult™ Osteogenic Differentiation Kit, or MesenCult™-ACF Chondrogenic Differentiation Kit, respectively. Similar results were observed with BM-derived MSCs.

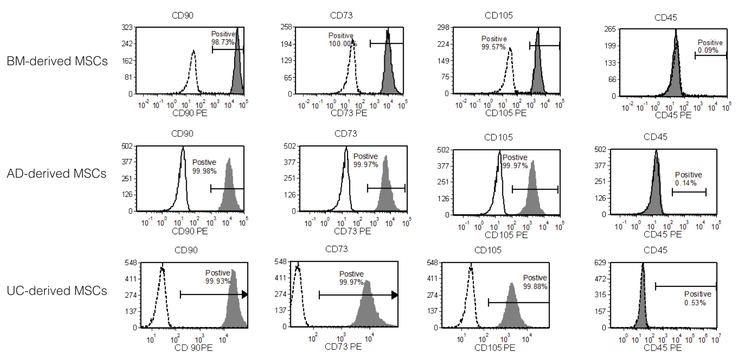


FIGURE 5. BM-, AD-, and UC-Derived MSCs Cultured in MesenCult™-ACF Plus Medium Express MSC Markers and Lack Expression of the Hematopoietic Marker CD45

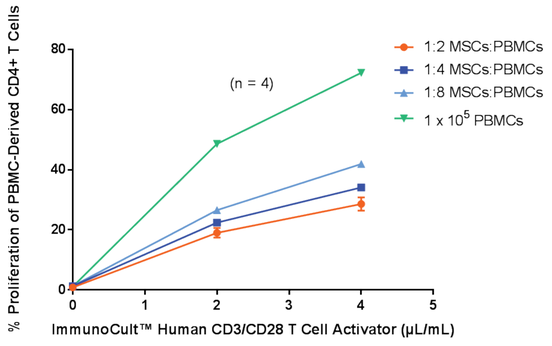


FIGURE 6. BM-MSCs Cultured in MesenCult™-ACF Plus Medium Suppress the Proliferation of PBMC-Derived CD4+ T Cells in a Dose-Dependent Manner

Summary

- MesenCult™-ACF Plus Medium is an improved culture medium for optimal derivation and expansion of BM- and AD-derived MSCs under complete ACF conditions
- MesenCult™-ACF Plus Medium also supports robust expansion of UC-derived MSCs
- BM- and AD-derived MSCs cultured in MesenCult™-ACF Plus Medium can differentiate robustly into adipocytes, osteogenic cells, and chondrocytes in vitro
- BM-derived MSCs cultured in MesenCult™-ACF Plus Medium are able to suppress CD4+ T cell proliferation in a cell concentration-dependent manner
- The MesenCult™-ACF Plus culture system provides a complete ACF workflow for the efficient derivation and expansion of different tissue-derived MSCs