

EXPANSION AND IMMUNOSUPPRESSIVE ABILITY OF MESENCHYMAL PROGENITOR CELLS FROM HUMAN BONE MARROW CULTURED IN A NOVEL SERUM AND ANIMAL-FREE CULTURE MEDIUM

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Introduction

Human mesenchymal progenitor cells (MPCs) are a phenotypically and functionally heterogeneous cell population typically isolated from bone marrow aspirates. Given the innate ability of these cells to give rise to multiple tissue types including bone, fat and cartilage, as well as their efficacy in the modulation of immune disorders such as graft versus host disease (GVHD), there is considerable interest in utilizing MPCs in a broad repertoire of cell-based therapies for the treatment of human disease.

MPCs are typically cultured in medium containing fetal bovine serum, which is a cause for concern when the cells are to be used in clinical applications, as potential incorporation of bovine derived proteins may lead to immunogenicity and rejection in transplant recipients. Traditional serum-containing medium is also problematic in that it facilitates not only the expansion of MPCs but also of contaminating plastic adherent hematopoietic cell populations which may persist for several passages.

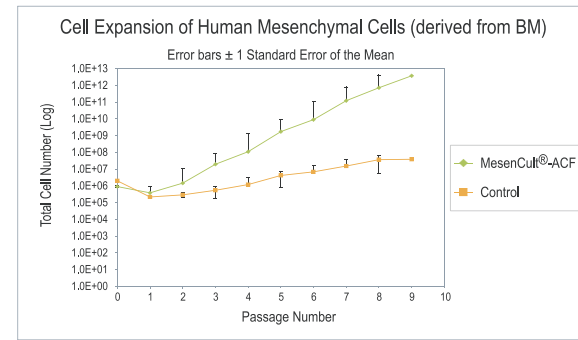
We have developed a humanized, serum and animal-component free medium (MesenCult®-ACF) formulation which promotes superior clonogenic growth and supports long-term expansion whilst maintaining multi-lineage differentiation potential of human bone-marrow derived mesenchymal cells in culture. Importantly, MPCs cultured in MesenCult®-ACF are essentially devoid of all hematopoietic contaminants at first passage.

Materials and Methods

- Culture plates were coated with MesenCult®-ACF Attachment substrate (Cat.#05425), which is essential for culturing MPCs in MesenCult®-ACF medium (Cat.#05420).
- Clonogenic growth of MPCs was analyzed by plating cells at low densities in 6-well plates. BM-derived mononuclear cells were plated at concentrations of 1.5 - 5.0 x 10⁵ cells/well in MesenCult®-ACF or at 2.5 x 10⁵ to 1 x 10⁶ cells/well in serum-containing medium (Control). Culture expanded MPCs were plated at concentrations of 25 - 200 cells/well in MesenCult®-ACF or at 100 to 1250 cells/well in Control medium. After 8 - 14 days of culture, MPCs were stained with Giemsa stain and CFU-F derived colonies were enumerated.
- Expansion assay was performed by plating Primary BM mononuclear cells in MesenCult®-ACF between 3.0 - 7.0 x 10⁴ cells/cm² and 1.5 - 4 x 10³ cells/cm² for passaged cells. Primary BM mononuclear cells in Control medium were plated at 1 x 10⁵ - 4 x 10⁵ cells/cm² and 5 x 10³ - 1 x 10⁴ for passaged cells. At each passage MPCs were dissociated with MesenCult®-Dissociation kit (Cat.# 05426), which is essential for subculture of cells in MesenCult®-ACF.
- Differentiation assays of MPCs into adipocytes, osteogenic cells and chondrocytes were performed with cells cultured at various passage number. Oil Red O, Alizarin Red and Alcian Blue stains were used to confirm the presence of adipocytes, osteogenic cells and chondrocytes.
- Cell surface phenotype of culture expanded cells in both MesenCult®-ACF and Control was analyzed by FACS at P1, P3, P5, P7 and P9.
- Human T-cells were isolated from PBMC's (EasySep® Human T-cell negative enrichment kit, Cat. #19051) and labeled with CFSE. MPCs generated in MesenCult®-ACF or Control were incubated with mitomycin C prior to co-culture with T-cells. T-cell - MPCs co-cultures were performed in serum-free XIVO-15 media supplemented with 100U/mL recombinant human IL-2. T-cell activation was induced by the addition of antibodies against human CD3e, CD28 and CD2. CFSE labeled T-cells (2x10⁵) were cultured with 1x10⁵ or 1.25x10⁴ MPCs. On day 3 and 7, cells stained with fluorochrome conjugated anti-human CD45 and propidium iodide (PI) were analyzed on a BD FACSCalibur.

Results

Fig. 1. Expansion of Human BM-derived MPCs cultured in MesenCult®-ACF and FBS containing medium (Control)



MPCs were cultured for 9 passages in MesenCult®-ACF with an average cell expansion of 8.5 ± 1.4 fold; mean ± SD (n=3), at each subculture. In comparison, cells cultured in a range of commercially available serum-containing media or in Control medium exhibited a comparatively lower proliferation rate. The average expansion of cells in Control was 2.7 ± 0.8 fold; mean ± SD (n=3), at each subculture.

Table 1. CFU-F-derived colony frequency and size (average from all seeding densities) of MPCs cultured in MesenCult®-ACF and Control medium

CFU-F/10 ⁶ BM MNCs Mean ± SD; n = 6		CFU-F Size (mm) Average diameter ± SD; n = 3 (Range)	
Control	MesenCult®-ACF	Control	MesenCult®-ACF
76 ± 44	88 ± 54	2.8 ± 0.99 (1.5 - 6)	5.7 ± 0.3 (2.3 - 11)

Frequency of CFU-F derived colony in both media was comparable. However, MesenCult®-ACF generated colonies that were on average twice as large as those cultured in serum-containing medium.

Fig. 2. CFU-F frequency of passaged cells cultured in MesenCult®-ACF and Control medium

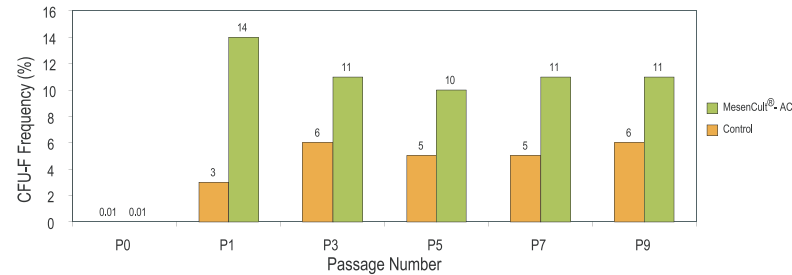
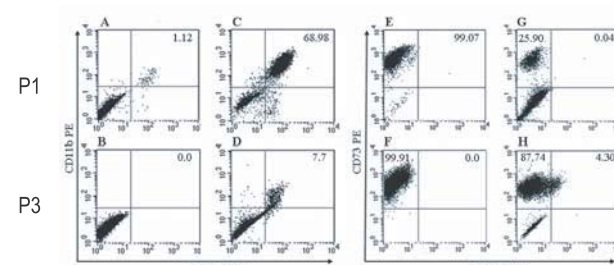


Fig. 3. MPCs cultured in MesenCult®-ACF (A, B) contain less hematopoietic contamination compared to MPCs cultured in Control medium (C, D) at P1 and P3 culture phase (P1=First Passage).



Cells cultured in MesenCult®-ACF contain 1% and 0% double positive cells (CD45 and CD11b) at P1 and P3, respectively. Cells cultured in Control medium contain 69% and 8% double positive cells (CD45 and CD11b) at P1 and P3, respectively. Staining of cells in MesenCult®-ACF (E, F) and Control medium (G, H) for CD73 gave 99% (P1) and 99.9% (P3) positive cells and 26% (P1) and 88% (P3) positive cells, respectively.

Fig. 4. Differentiation of Human BM-derived MPCs expanded in MesenCult®-ACF

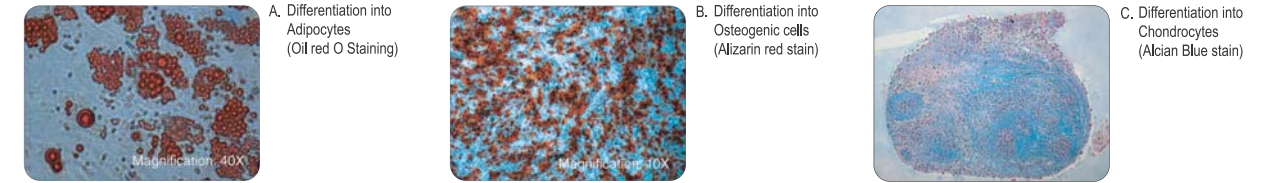


Fig. 5. FACS analysis of BM-derived MPCs (P3) in MesenCult®-ACF (A) and Control (B) media.

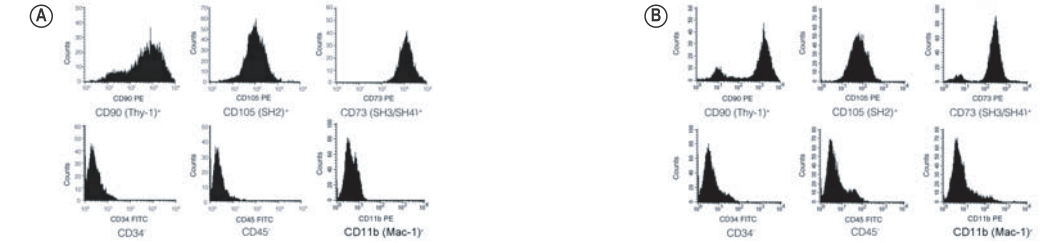
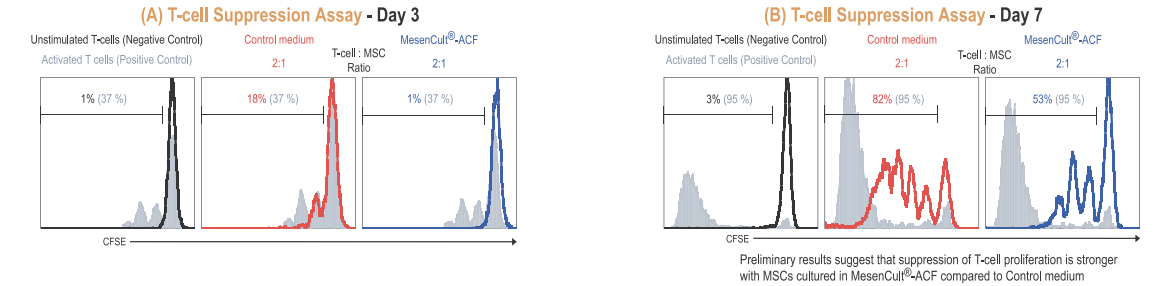


Fig. 6. T-cell suppression assay at day 3 (A) and day 7 (B)



Conclusions

- MesenCult®-ACF is a defined serum-free formulation with no animal-derived components, which promotes superior clonogenic growth and expansion of BM-derived and cultured MPCs.
- MPCs cultured in MesenCult®-ACF have less hematopoietic contamination at early passage compared to serum-based Control medium.
- MPCs cultured in MesenCult®-ACF showed greater expansion (>8 passages) compared to serum-based Control medium while maintaining multi-lineage differentiation capacity.
- MPCs cultured in MesenCult®-ACF have a surface marker phenotype characteristics of MPCs: CD90⁺, CD105⁺, CD73⁺, CD34⁻, CD11b⁻ and CD45⁻.
- MPCs cultured in MesenCult®-ACF showed stronger suppression of T-cell proliferation compared to MPCs cultured in Control medium.



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